NEW SERIES VOL. 22 NO. 86 APRIL 1958

ANNALS OF BOTANY

EDITED BY
W. H. PEARSALL

ASSISTED BY
F. G. GREGORY
T. M. HARRIS
C. T. INGOLD
K. MATHER

UNIVERSITY OF ILLINOIS
LIBRARY
JUN 16 1958
CHICAGO

OXFORD: AT THE CLARENDON PRESS
Price 22s. net

CONTENTS

PAGE

INGOLD, C. T. On Light-stimulated Spore Discharge in *Sordaria*. With four Figures in the Text 129

DELAP, ANNE V., and FORD, ELSIE M. Studies in the Nutrition of Apple Rootstocks. With eight Figures in the Text 137

ROBERTSON, N. F. Observations of the Effect of Water on the Hyphal Apices of *Fusarium oxysporum*. With four Figures in the Text 159

POLJAKOFF-MAYBER, A., MAYER, A. M., and ZACKS, S. Interaction in Growth and Germination between Thiourea and Indolyl Acetic Acid 175

DAVIDSON, D. The Irradiation of Dividing Cells: Changes in Sensitivity to X-Rays during Mitosis. With three Figures in the Text 183

CHALONER, W. G. Isolated Megaspore Tetrads of *Stauropteris burntislandica*. With Plate and one Figure in the Text 197

NAYLOR, MARGARET. The Cytology of *Halidrys siliquosa* (L) Lyngb. With four Plates . 205

BUTLER, GILLIAN M. The Development and Behaviour of Mycelial Strands in *Merulius lacrymans* (Wulf.) Fr. With seven Figures in the Text 219

PLUNKETT, B. E. Translocation and Pileus Formation in *Polyporus brumalis*. With Plate . 237

HUMPHRIES, E. C. Effect of Removal of a part of the Root System on the Subsequent Growth of the Root and Shoot. With four Figures in the Text 251

HUSSEY, G. An Analysis of the Factors Controlling the Germination of the Seed of the Oil Palm, *Elaeis guineensis* (Jacq.). With two Plates and six Figures in the Text . . 259

WARREN WILSON, J., and WADSWORTH, R. M. The Effect of Wind Speed on Assimilation Rate—a Re-assessment. With two Figures in the Text 285

ROACH, W. A. A Machine for Disintegrating Living Woody Shoots. With Plate . . 291

ROACH, W. A. A Continuous Low-temperature Vacuum Extractor. With Plate and one Figure in the Text 293

NOTICE TO CONTRIBUTORS

Contributors should address their papers to Prof. W. H. PEARSALL, Department of Botany, University College, London, W.C. 1.

Papers sent for publication *should be typewritten. They should include a short abstract and should conclude with a summary of the contents.* In view of the increasing numbers of manuscripts submitted, the Editors desire to impress upon contributors the importance of conciseness. Bodies of quantitative data, too extensive for complete publication, should be summarized for discussion and the originals deposited in the Archives established for this purpose in the British Museum (Natural History), South Kensington, S.W. 7. The Editors suggest that the longer papers should not exceed 10,000 words. Contributors will receive one proof in page. An allowance at the rate of ten shillings per sheet of sixteen pages is made for alterations in the proof (printers' errors excepted), and contributors will be responsible for any excess.

ILLUSTRATIONS. These where possible should be suitable for reproduction as line-blocks in the text. Where half-tone plates are required the figures should be planned so as to fill properly a 4to or an 8vo plate. The maximum space available for figures in a 4to plate is $8\frac{1}{4}\times 11\frac{1}{4}$ inches, in an 8vo plate $8\frac{1}{4}\times 5\frac{1}{4}$ inches. The lettering of figures, whether text-figures or those of plates, should be in pencil.

The Journal is published four times yearly by the publishers, Oxford University Press, Amen House, Warwick Square, London, E.C. 4, to whom subscriptions and all communications, other than editorial, should be addressed.

The subscription price for four numbers is 55*s*. (foreign postage, 1*s*. 6*d*. extra), single copy 22*s*. net. Cloth cases may be obtained, price 7*s*. 6*d*.

FIRST SERIES (VOLUMES 1–50)

Orders and inquiries, other than those from U.S.A., for copies of the first series (Volumes 1–50) of the Annals of Botany should be addressed to The Assistant Treasurer, Annals of Botany Company, Botany School, Cambridge, England. Messrs. J. S. Canner & Co., 46 Millmont Street, Boston 19, Mass., are the sole distributors in the U.S.A. and all American inquiries should be addressed to them.

Stocks of Volumes 1, 2, 19–25, 28, 30–34, 36–40 *are exhausted.* Most of the early volumes are in sheet form, unbound; and in a few cases the volumes are slightly imperfect.

The prices of the back numbers, including packing and postage, are £2. 10*s*. 0*d*. a volume, whether in the form of bound parts or separate sheets (half price if imperfect); 20*s*. a single part (bound); 10*s*. a single part (in sheets). Indexes at usual published prices.

Cheques, other than those from U.S.A., should be made payable to The Annals of Botany Company and sent to The Assistant Treasurer.

The publishers are signatories to the Fair Copying Declaration in respect of this journal. Details of the Declaration may be obtained from the office of the Royal Society upon application.

Books *on BOTANY*

AGRICULTURE and Allied Sciences of all publishers supplied from stock.

Catalogue of Books on Botany, Plant Breeding and Genetics post-free on request.

Scientific Lending Library

New Books and latest editions obtainable.
ANNUAL SUBSCRIPTION, TOWN OR COUNTRY
£1. 17*s*. 6*d*.

THE LIBRARY CATALOGUE revised to December 1956, containing a classified Index of Authors and Subjects.
To Subscribers £1. 5*s*. net, to non-subscribers £2. 2*s*. net, postage 2*s*.

Second-Hand Books

Select stock of recent editions of books on Botany &c. Books sought for and reported free of charge.

H. K. LEWIS & Co. Ltd.
LONDON: 136 Gower St., W.C. 1
Telephone: **EUSton 4282 (7 lines)**

QUARITCH
have now purchased the entire stock of books on
CACTI
and
SUCCULENTS
FROM HENRY CORK WHO HAS RETIRED FROM BUSINESS

A CATALOGUE OF BOOKS ON THESE SUBJECTS
is in active preparation and will be sent abroad and in Great Britain post free on receipt of 2s. P.O.

Agents for the publications of the British Museum South Kensington, Ray Society, Royal Horticultural Society, &c.

BERNARD QUARITCH Ltd.
11 Grafton Street, New Bond Street
London, W. 1

You will obtain much better sections with original
JUNG
Microtome Knives

There is a JUNG knife for each sectioning technique and for almost any microtome:
Jung, Leitz, MSE,
Spencer, Reichert,
Cambridge . . .
Other knives will be made to order.

Please write for leaflet 456/Rev with latest prices

GLEN CRESTON LTD.
41 Church Road, Stanmore, Middlesex
Telephone: GRIMSDYKE 3468

BOTANICAL BOOKS

New, Second-hand
Old and Rare

Catalogues on request

WHELDON & WESLEY, LTD.
LYTTON LODGE
Codicote, Near Hitchin
Herts.
Telephone: Codicote 370

Libraries and small parcels of books purchased for cash

ECOLOGY

Official Publication of the Ecological Society of America
Continuing the Plant World

Editors:

Botany: ALTON A. LINDSEY
Purdue University
Lafayette, Indiana

Zoology: DR. LAMONT C. COLE
Cornell University
Ithaca
N.Y., U.S.A.

Established 1920 *Quarterly*

Subscriptions, 56*s*. 6*d*. a year (Foreign postage 4*s*. 6*d*. extra) for complete volumes (January to October, inclusive). Single copies 15*s*. each, post paid. Back volumes, as available, 60*s*. Postage extra on quantity shipments.

DUKE UNIVERSITY PRESS

Address all subscriptions or orders for special issues to

CAMBRIDGE UNIVERSITY PRESS

200 EUSTON ROAD, LONDON, N.W. 1

MICHROME STAINS

and Biological Reagents

Carminic Acid
Cedarwood Oil
Celloidin
Chlorazol Azurin
Chlorazol Black
Chlorazol Paper Brown
Cytase
Fast Green
Haematoxylin
Janus Green B
Lacmoid
Lignin Pink
Lipase
Methyl Green
Pyronin
Sudan Black
Toluidine Blue
Urease
Wright Stain, &c.

AQUAMOUNT: synthetic, neutral, colourless aqueous mountant

CRISTALITE & CLEARMOUNT: synthetic, colourless, neutral mountants

OPTOIL: synthetic, non-sticky immersion oil

55-page Price List available on request

Outfits & Tablets for Water Analysis

EDWARD GURR, LTD.

42 Upper Richmond Road West
LONDON, S.W. 14

Telephones: PROspect 8051 and 7606

Service, uniform high quality, reliability, and immediate delivery

Ready early 1958: **'Methods of ANALYTICAL HISTOLOGY & HISTOCHEMISTRY' by Edward Gurr. First edition, about 475 Royal 8vo pages, approx. 65s. net**

Ready April 1958:
MICROSCOPIC STAINING TECHNIQUES, No. 4, about 90 pages, price about 6s.

HEFFER'S

BOOKS ON BOTANY IN ALL LANGUAGES

★

Catalogues available free; books and learned journals bought

★

W. HEFFER & SONS LTD.

Petty Cury · Cambridge

Annals of Botany Memoirs, No. 1

A MONOGRAPH OF CLAVARIA AND ALLIED GENERA

By E. J. H. CORNER

Royal 8vo, 756 pages, with 16 coloured plates and 298 text-figures

£5. 5s. net

This is the first volume of a new series to be published by the Annals of Botany Company, under the general title of *Annals of Botany Memoirs*.

The book embodies a very large amount of research of the highest quality, which it is most important to publish for the information of other investigators of the Fungi. In addition to its great taxonomic interest and value the monograph contains detailed accounts of the developmental morphology of these fungi, in which the author has thrown much new light on the way in which their fruit-bodies are built up. No other group of the Higher Fungi has yet been investigated on a world-wide basis in this manner. The author's views on the morphology of the Clavarias and allied genera are certain to have great influence on the interpretation of the structure and classification of the Higher Fungi generally. The book, therefore, is an important contribution to science.

OXFORD UNIVERSITY PRESS

Routine Tests . . .
or Research Projects

There is an MSE Centrifuge Model, with or without refrigeration, exactly suited to the application.

Ask for MSE Publication 888 AB, which gives details of all MSE models.

MSE

MEASURING & SCIENTIFIC EQUIPMENT LIMITED
Spenser Street, London, S.W.1 · Tel.: VICtoria 5426

The Grasses of Barbados

By J. A. ALLAN

Because of their great economic value, more importance than the immediate value to botany and agriculture attaches to the ready identification of the grasses of a region and to the means of distinguishing between superficially similar species. In this systematic attack on the problem as it affects Barbados, Mr. Allan describes some 80 species and has provided a useful key to their vegetative character. Nearly half the work is devoted to full-page detailed illustrations of 56 species.

7s. 6d. (*post 6d.*)

HMSO

from the Government Bookshops in London, Edinburgh, Manchester, Birmingham, Cardiff, Bristol, and Belfast; or through any bookseller

On Light-stimulated Spore Discharge in *Sordaria*

BY

C. T. INGOLD

(*Birkbeck College, London*)

With four Figures in the Text

ABSTRACT

A rough action-spectrum curve (in the range 400–600 mμ) for light-stimulated spore discharge in *Sordaria fimicola* is compared with the absorption-spectrum curve of an ethyl-alcohol extract of the fungus. The two curves show some agreement. A mutant lacking black pigment (probably melanin) is shown to respond to light in its spore discharge as does the normal, but evidence is given that it is more sensitive to light. It seems that the melanin in the perithecial wall of the normal strain acts as a neutral filter reducing the effectiveness of the incident light.

INTRODUCTION

IN a previous paper (Ingold and Dring, 1957) it was pointed out that although *Sordaria fimicola* can mature its perithecia and discharge its spores in total darkness, transfer of active perithecia from dark to light always leads to a marked rise in the rate of spore discharge. Evidence was also given to show that in this reaction it is blue rays which are particularly effective whilst red light has little or no effect.

This paper is concerned with a further analysis of the effect on discharge of light of different wave-lengths with some reference to the problem of the pigment or pigments which are presumably concerned in the perception of the light stimulus.

QUALITY OF LIGHT IN RELATION TO DISCHARGE

An attempt was made to construct an approximate action-spectrum of light-stimulated discharge. In the previous work, referred to above, substantially equal intensities of light of different colours were obtained by using a strong source of white light and passing this through appropriately coloured gelatine filters in association with suitable 'neutral' filters and a sheet of infra-red-absorbing glass. The intensity was measured by means of a highly sensitive thermophile. However, with this type of set-up it is difficult to make accurate measurements unless comparatively high light intensities are used and it is not easy to obtain these unless the colour-filters employed have a rather wide wave-band of transmission. In the present investigation, therefore, it was decided to measure intensities of light in the various regions of the spectrum by using a selenium photo-cell. However, in such a cell the region of reasonably high sensitivity is limited and it is not very useful in measuring light intensities in the red regions of the spectrum. The selenium cell has its maximum sensitivity at 570 mμ in the greenish-yellow. The sensitivity sinks to

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

60 per cent. of the maximum at 400 mμ on the violet side and 635 mμ on the orange side. This photo-cell can be effectively employed to measure light intensity over the range 400–635 mμ, if allowance is made for change of sensitivity with wave-length. Two 'daylight' fluorescent tubes were used as a source of the light, which to reach the fungus passed through a coloured gelatine filter of narrow spectral transmission. This was used in conjunction with an infra-red-absorbing filter (Chance ON 20) and, where necessary, with an appropriate 'neutral' filter. By using graded neutral filters the light of different colours could be roughly adjusted to approaching equality. The set-up used for determining spore discharge rate was the black 'perspex' box described in the earlier paper. This was placed in an incubator at 21° C. illuminated by fluorescent tubes from above. By moving the box up or down within the incubator, the light intensity could be increased or decreased to some extent, and in this way fine adjustment of the intensity of the light, incident on the fungus, to more exact equality in the different spectral regions was made.

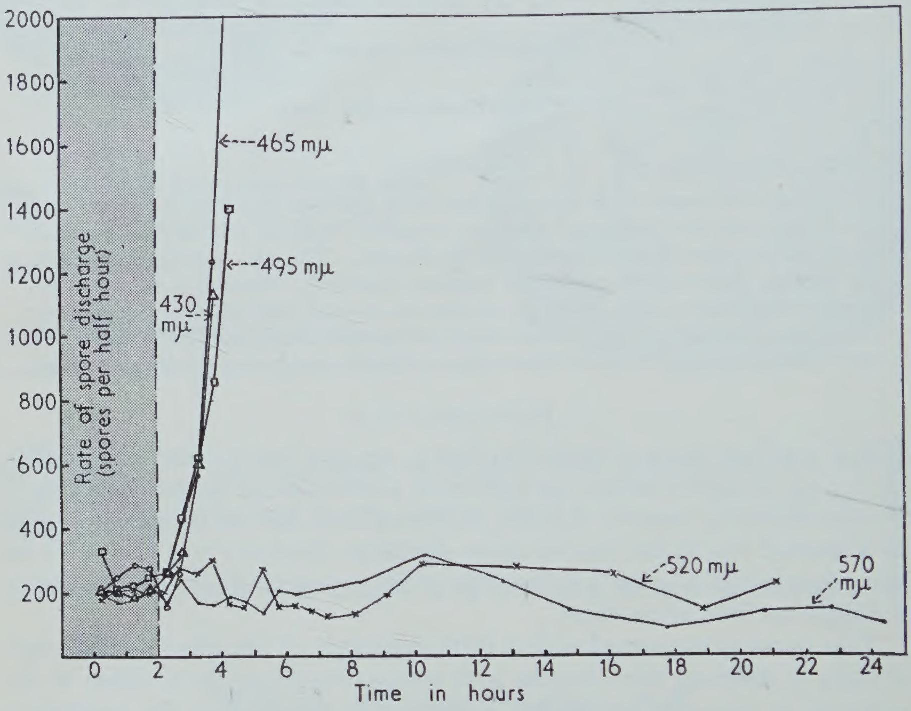


FIG. 1. Rate of spore discharge in *Sordaria* plotted against time for five cultures in dark and after transfer to light of different colours but of the same intensity. The number against each graph shows the wave-length of maximum transmission for the light-filter used. One point of the graph labelled 465 mμ is above the frame of the diagram. Temperature 21° C.

In these experiments the absolute intesnity of the light used was low, being of the order of magnitude of 1·0–1·5 f.c.

The fungus was grown on yeast-extract-filter-paper agar in 2·5×2·5 cm. specimen tubes as previously described. Cultures were grown in the light incubators (50–100 f.c.) at about 21° C. Before starting an experiment the culture was placed in the dark overnight and the rate of discharge was then determined in the dark before giving the light treatment. If the rate in the dark was not reasonably high and fairly consistent the culture was discarded.

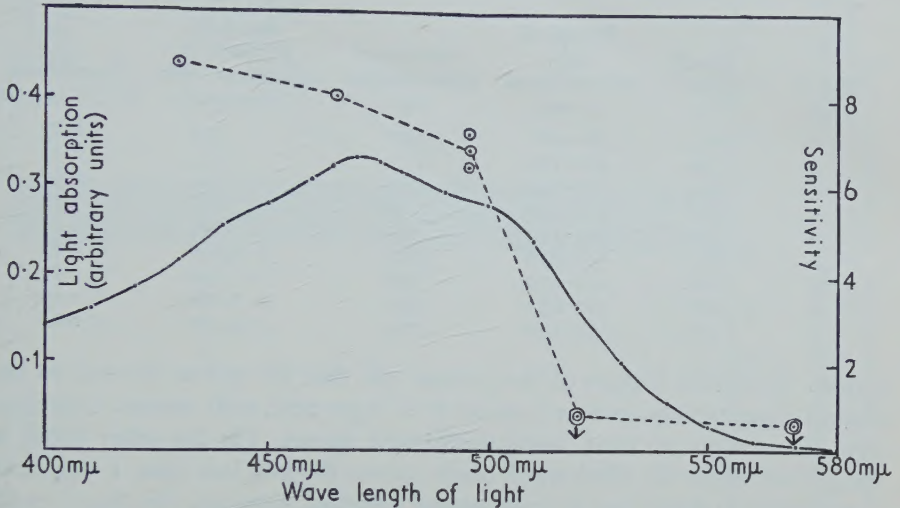


FIG. 2. Absorption-spectrum curve of 95 per cent. ethyl-alcohol extract of *Sordaria*, and sensitivity curve (dashed line) of spore discharge to light of different wave-lengths. For sensitivity data see Table 1.

The results of an experiment at each of the wave-length bands tested are shown in Fig. 1. It is clear that stimulation of discharge is considerable in blue regions of the spectrum, but there is no evidence of an effect in green or yellow light. For the sake of comparison of one experiment with another a measure of the sensitivity of the fungus to light is desirable. In the dark there is normally a fairly steady rate of discharge. This rate (spores discharged per half-hour) was averaged over a 2-hour period. Then on transfer to light the time in minutes (T) for the rate to be increased five-fold was determined from the graphs (Fig. 1). The value of sensitivity, $1/T$ (for convenience multiplied by 10,000) is recorded in Table 1 which also shows the spectral range of the filters and the wave-length of maximum transmission for each. The sensitivity figures are plotted in Fig. 2. together with the absorption-spectrum of an ethyl-alcohol extract of the fungus prepared as described previously (Ingold and Dring, 1957).

LIGHT AND DISCHARGE IN A MUTANT LACKING MELANIC PIGMENT

Whilst the investigation was in progress a mutant of the strain of *S. fimicola* which was being used appeared in culture and was duly isolated. This had orange-yellow perithecia and colourless spores. It would seem that the mutation involved the loss of the ability to form black pigment (probably melanin) responsible both for the colour of the perithecial wall and of the spores. A curious feature of the mutant was that the spores showed an enhanced capacity to germinate in water or in 2 per cent. malt extract. Germination exceeded 90 per cent. using discharged spores. On the other hand, in the parent strain the discharged black spores showed less than 1 per cent. germination in water and in malt extract solution, although this figure could be increased to over 90 per cent. by the addition of acetate (Bretzloff, 1954). It was felt that the mutant might differ from the parent strain in tyrosinase activity, since a lack of that enzyme would explain the failure to produce melanin from tyrosine. However, it was found that extracts of both parent and mutant strains had a strong tyrosinase activity.

TABLE 1

Rate of Spore Discharge and Light-quality

No. of expt.	Ilford filter No.	Range of light transmission in mμ	Maximum transmission mμ	Time (T) for five-fold increase in discharge rate (minutes)	Sensitivity ($1/T \times 10{,}000$)
1	601	380–480	430	114	8·77
2	622	380–530	475	123	8·13
3	623	460–540	495	144	6·94
4	623	460–540	495	138	7·25
5	623	460–540	495	155	6·45
6	624	490–570	520	> 1,200	< 0·83
7	624	490–570	520	> 1,200	< 0·83
8	626	550–630	570	> 1,500	< 0·66
9	626	550–630	570	> 1,500	< 0·66

It was clearly of interest to see if the melanin-free strain responded to light as did the normal. If it were to, it would be evident that the melanin was not the photo-receptor or, at any rate, not the sole photo-receptor concerned with light-stimulated discharge.

It was at once apparent that the mutant reacted to light in the same general manner as did the normal, but in the early experiments it was noticed that the response to light seemed to occur with less delay than in the normal. This problem was further investigated.

The black-box method of studying the rate of spore discharge was employed, but two specimen-tube cultures, one mutant and one normal, were placed side by side in the box and the slide carried by the slide-holder had two etched grids (each a square centimetre subdivided into square millimetres) so arranged to receive the spores shot from the two cultures. Cultures raised in continuous light at about 21° C. were used and placed in the dark box overnight before the start of an experiment. Next day the rate of discharge was followed in the dark for 1 and 2 hours before subjecting the fungus to light. The results of three experiments are shown in Fig. 3.

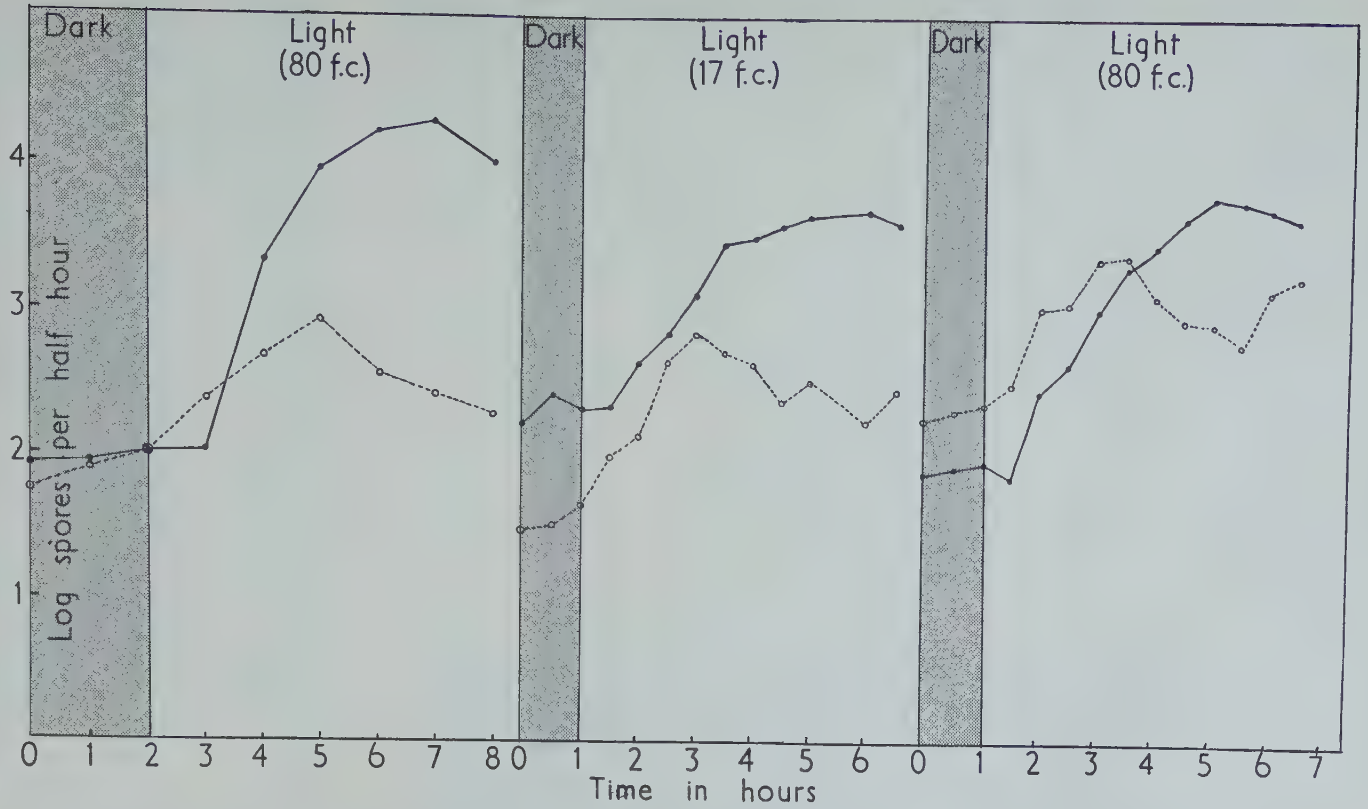


FIG. 3. Log. rate of spore discharge in *Sordaria* plotted against time (for three pairs of cultures) in the dark and after transfer to light. The solid line with black points in each pair is the normal; the dashed line with white points the melanin-free mutant. Cultures were kept in the dark overnight before the start of an experiment. Temperature 21° C.

Two general points emerge. First, when a 'normal' culture is transferred to light there is a brief lag period of half an hour or so before the effect of the illumination on discharge rate is shown, but in the mutant the effect is almost immediate. This can be seen on examining the three pairs of graphs in Fig. 3. In each pair the upward slope of the curve immediately following illumination is greater in the mutant than in the normal. The second point is that the peak rate following illumination is reached earlier in the mutant. Using the normal fungus with drastically different light intensities it is found that higher light intensity gives quicker response and the peak rate comes sooner. This may be illustrated by considering a particular experiment recorded in Fig. 4. In this the rate of discharge in a normal culture (raised in the light and then kept overnight in the dark) was followed in the dark and then after transfer to light of 50 f.c. The normal rise in discharge rate to a peak followed by a fall was

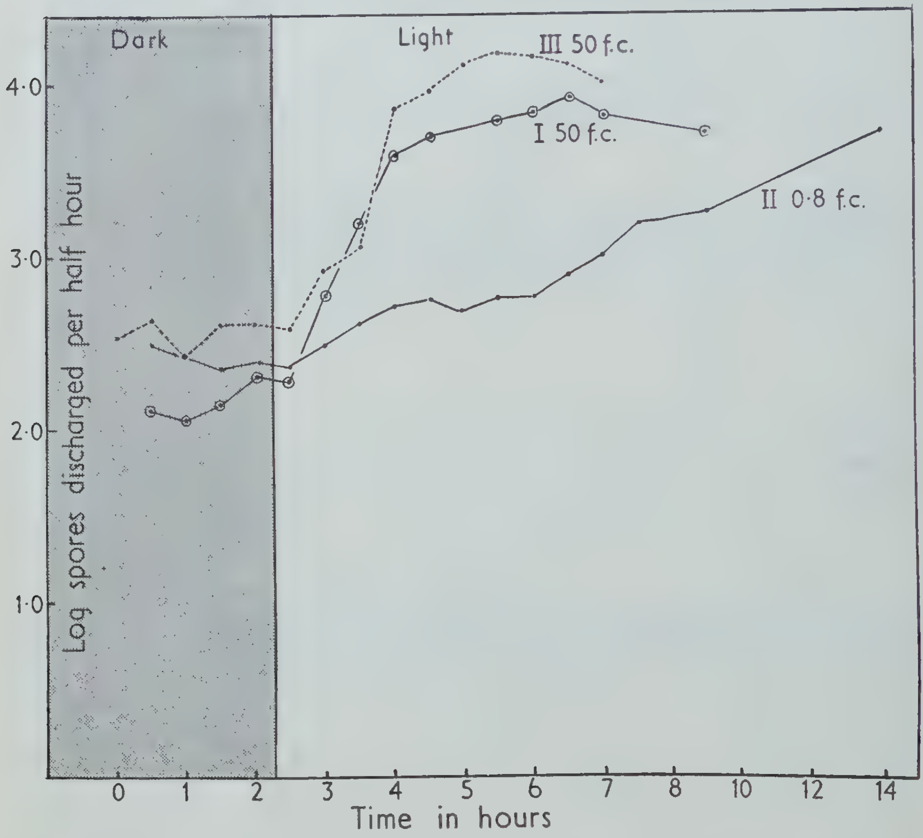


FIG. 4. Log rate of spore discharge in *Sordaria* plotted against time for a single culture on three successive days (I, II, and III) from about 10 a.m. onwards on each day. For the night period before the start of the experiment the culture was in the dark and also during the night periods between the first and second, and the second and third days. Temperature 21° C.

observed. The culture was returned to darkness overnight. Next day the process was repeated but light of a much lower intensity (0·8 f.c.) was used. With this intensity the rise was much slower on illumination and the peak was not reached even after 12 hours. The culture was then returned to darkness and the following day the procedure was repeated, but the higher light intensity was used. The performance of the first day was very closely followed.

DISCUSSION

So far the evidence is fairly consistent with the theory that the orange pigment or pigment complex is concerned with photo-reception in connexion with the stimulation of spore discharge by light. It is true that the rough action spectrum shown in Fig. 2 fits the absorption-spectrum curve of the pigment only in a very general manner, but this may well be due to lack of exactness in the measurement of sensitivity. Further, as pointed out previously (Ingold and Dring, 1957) a culture raised in the dark responds as quickly and as strongly to illumination as one which has been raised in the light and transferred to the dark the night before the experiment. Yet the former culture has very much less yellow-orange pigment than the latter. Nevertheless this pigment is not wholly absent from a culture grown in the dark and there is, perhaps, sufficient present for adequate absorption of light. The evidence obtained by comparing the melanin-free mutant with the normal suggests that the effect of the dark pigment, situated in the outer layer of the perithecial wall, is merely to reduce the effectiveness of the incident light in stimulating discharge.

The significance of the yellow-orange pigment in light-stimulated spore discharge could be put conclusively to the test if a completely albino mutant could be produced. Attempts, however, to obtain such a strain from the melanin-free mutant have so far failed.

I am indebted to Vivienne Dring for carrying out much of the experimental work and to Dr. R. A. Shaw for the absorption spectrum reproduced in Fig. 2.

LITERATURE CITED

BRETZLOFF, C. W., 1954: The Growth and Fruiting of *Sordaria fimicola*. Amer. J. Bot., **41**, 58–67.

INGOLD, C. T., and DRING, V. J., 1957: An Analysis of Spore Discharge in *Sordaria*. Ann. Bot., N.S., Lond., **21**, 465–77.

Studies in the Nutrition of Apple Rootstocks

1. Effects of Deficiencies of Iron and Magnesium on Growth

BY

ANNE V. DELAP AND ELSIE M. FORD

(*East Malling Research Station*)

With eight Figures in the Text

ABSTRACT

Plants of the apple rootstock M.VII were grown for a single season by spraying their roots continuously with nutrient solutions: (*a*) complete, (*b*) low iron, (*c*) low magnesium, and (*d*) low iron and low magnesium. Detailed records were taken throughout the season of fresh weight, shoot length, diameter, and leaf area, while the fresh and dry weights of component parts were determined on harvested samples.

The leaves of the iron-deficient plants were chlorotic and had a lower dry weight and higher water content per unit area than those of the control plants. Growth and net assimilation rate were both reduced but the distribution of assimilates was similar to that in the controls.

Total growth was also markedly reduced by the low-magnesium treatment, but the leaf symptoms were different and the plants were morphologically distinct from the control plants. Very little chlorosis occurred, but necrosis and severe defoliation, progressing up the shoot, reduced the leaf area ratio and probably largely accounted for the low net assimilation rate and relative growth rate. The diameter of the shoot was affected much more than its length and the shoot/root ratio was greatly increased.

Plants deficient in both iron and magnesium showed less defoliation than when magnesium alone was low. More dry matter accumulated in the old stem and less in the new shoot than would have occurred with a simple combination of the single deficiencies.

INTRODUCTION

SINCE Sachs first grew plants in water to which nutrients had been added, much work has been done on the effects of deficiencies of essential minerals. The approach has ranged from the purely empirical study in the field to the biochemical investigation in the laboratory.

Methods of detailed growth analysis have been applied to the study of mineral nutrition of plants by Gregory and Richards (1929) and others in the series of papers under the general title of 'Physiological Studies in Plant Nutrition', also, for example, by Watson (1947), Blackman and Rutter (1948), Morton and Watson (1948), and Ruck and Bolas (1954). This paper reports the use of these techniques in a study of the effects of varying the iron and magnesium supply to apple rootstocks under controlled conditions. The subject is of special interest in that both magnesium deficiency and lime-induced iron deficiency are common nutrient disorders of fruit crops in the field.

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

The literature on iron nutrition of plants has been reviewed by Brown (1956) and Speirs (1954) and that on magnesium nutrition by Krackenberger and Peterson (1954) and Zimmerman (1947). Sideris and Young (1956) have studied growth and metabolism of pineapple in relation to iron and nitrogen supply, and Smith, Reuther, and Scudder (1953) have compared the growth and cropping of magnesium-deficient and of healthy Valencia orange-trees. Wallace (1951) has described and illustrated deficiency symptoms in various crops in detail, and has examined the effects of magnesium deficiency on apple-trees in sand culture and in the field (Wallace, 1930, 1939, 1940).

METHODS

Apple-plants (one-year rooted shoots from stoolbeds of M.VII rootstock) were grown for a single season, sixteen plants being supported in each of sixteen containers. The roots were kept in darkness by close-fitting lids and subjected to a continuous spray of nutrient solution, each container having an independent supply (Roach *et al.*, 1957).

The nutrient solution was not recirculated and large volumes of purified water were therefore required and were obtained by adding lime water and ammonium dihydrogen phosphate to tap water (Roach *et al.*, 1957). The resulting flocculent precipitate carried down most of the impurities leaving an iron and magnesium content of less than 0·01 and 3 p.p.m. respectively. The pH of this water was adjusted to 5·5 by the addition of nitric acid and nutrients were added according to treatment.

Treatments

The treatments were:

(1) Complete nutrient solution
(2) Low iron
(3) Low magnesium
(4) Low iron, low magnesium

The complete nutrient solution was made up from concentrates of KH_2PO_4; KNO_3; $Ca(NO_3)_2, 4H_2O$; $MnSO_4, 4H_2O$; $CuSO_4, 5H_2O$; $ZnSO_4, 7H_2O$; H_3BO_3; Fe–EDTA complex, and $MgSO_4, 7H_2O$, to give the following final composition with a pH of approximately 5·5:

N	185	p.p.m.	Mn	0·5	p.p.m.
P	20	,,	Zn	0·2	,,
K	200	,,	Cu	0·1	,,
Ca	200	,,	B	0·5	,,
S	64	,,	Fe	5·0	,,
Mg	48	,,			

In treatments (2) and (4) no iron was added and in treatments (3) and (4) no magnesium was added, giving an iron content of less than 0·016 p.p.m. and a magnesium content of less than 3·0 p.p.m. introduced as impurities in the chemicals and water.

The treatments were replicated four times and were randomized within each block of four containers.

Weinstein *et al.* (1956) showed that, although EDTA had a stimulating effect on the growth of lupin hypocotyls, the iron chelate at a concentration of 10^{-4} M had a slight depressing effect on growth. This concentration is similar to that used in these experiments but it was considered that any error introduced by the presence of the chelated complex in the full nutrient solution and its absence from the low-iron treatments was negligible compared with the main results expressed here.

Plant material

Stoolbed material was used as it provided a source of small, uniform, conveniently handled plants. Type M.VII was chosen because it was of medium vigour, its shoots developed few lateral growths and it produced flat leaves, all of which made easier the estimations of shoot length and leaf area.

A selection of 600 plants was made from approximately 2,000 rooted shoots on a basis of weight, length, straightness of stem, and absence of damage. All roots were removed and any soil was washed off; the plants were stored at 37° F. until required. At 'planting' time each shoot was pruned to one-third its original length to deplete reserves, all buds were removed except the distal three and the plants were weighed individually. Twice the number of plants required were placed in each container. Following a preliminary experiment which was begun on 8 June 1953, the main experiment was carried out in 1954 when the plants were put out in the containers on 6 April. They were supplied with the full nutrient solution without the addition of iron and magnesium for 10 weeks, until 15 June. This initial period of growth served to deplete the reserves of iron and magnesium in the plant and provided data with which to adjust corresponding later records by means of analyses of covariance thereby reducing their variability. During this period, while the three shoots at the apex were still less than 1 inch long, two were removed leaving that which conformed most nearly to the average. (This shoot is hereafter referred to as the 'new shoot' and the parent rootstock from which it and the roots grew is termed the 'old stem'.) Before treatments were started the length of the new shoot was measured and the more variable half of the population was discarded, leaving 256 plants.

Measurements

Measured group of plants: Fresh weights and new shoot lengths of nine of the plants in each container were recorded at intervals of about a fortnight throughout the season. This gave a total of thirty-six plants per treatment. The diameters of the old stems of these plants were measured three times. Leaf areas were estimated on six of the above nine plants at approximately fortnightly intervals, by the method of counting squares; a piece of Perspex marked in square centimetres was placed over the leaf and the number of whole, three-quarter, half, and quarter squares occupied by the leaf was counted. At the same time a record was kept of the area of necrosis occurring on each leaf. Any other symptoms were also noted.

Harvested group of plants: One plant per container (i.e. four per treatment) was harvested on each of seven occasions at intervals of approximately 3 weeks from 8 June, when treatments started, until 22 October. New shoot length, leaf symptoms, and leaf areas were measured and the fresh weights of the whole plant, of the new shoot, and of individual leaves with their petioles were recorded. These samples were cleaned by washing (Mason, 1953) and were dried at 80° C. The dry weights of the bark and wood of the new shoot, leaves, petioles, roots, and old stem were determined and a total dry weight was obtained by adding these weights. The samples were subsequently analysed and these chemical data will be dealt with in another paper.

RESULTS

Growth effects in 1953 were similar to, but not as pronounced as, those in 1954 probably owing to the late planting date and slight contamination of the low-iron solutions with iron. As similar trends were found in both years only 1954 data are presented here.

The measured group of plants grew rather less than those in the harvested group, which were not handled during the season. This does not invalidate the results expressed here, since the relative treatment effects were not altered and comparisons are not made between the two groups of plants. Subsequent experience has justified the use of regression lines, based on data from the harvested group, to convert the fresh weights of the measured plants to dry weights for the purpose of calculating their relative growth rates and net assimilation rates.

Foliar Symptoms

Complete nutrition: Although no iron or magnesium was supplied to the plants during the preliminary period of 10 weeks, the control plants did not develop symptoms of iron deficiency and their leaves were a healthy dark green. Later in the season, however, a few lower leaves developed necrotic areas and an average of three per plant abscissed. These symptoms are attributed to slight magnesium deficiency (see below) but, since the plants grew well, it is not considered that they were seriously affected by this mild deficiency; large differences in growth occurred between the control plants and those in the low-magnesium treatment.

Iron deficiency: By 21 June the apical leaves of the iron-deficient plants were noticeably pale and often slightly bronzed. After a few days these leaves developed the characteristic symptoms of dark green veins on a pale green background. Subsequently formed leaves became increasingly more yellow, were brittle to the touch, and curled upwards from the midrib. Finally irregular, orange, necrotic patches developed on the apical leaves, but there was little defoliation. Leaves at the base of the shoot remained green throughout the season.

Magnesium deficiency: Symptoms became apparent by 28 June when a few leaves in the middle of the shoot showed slight purpling of the margins,

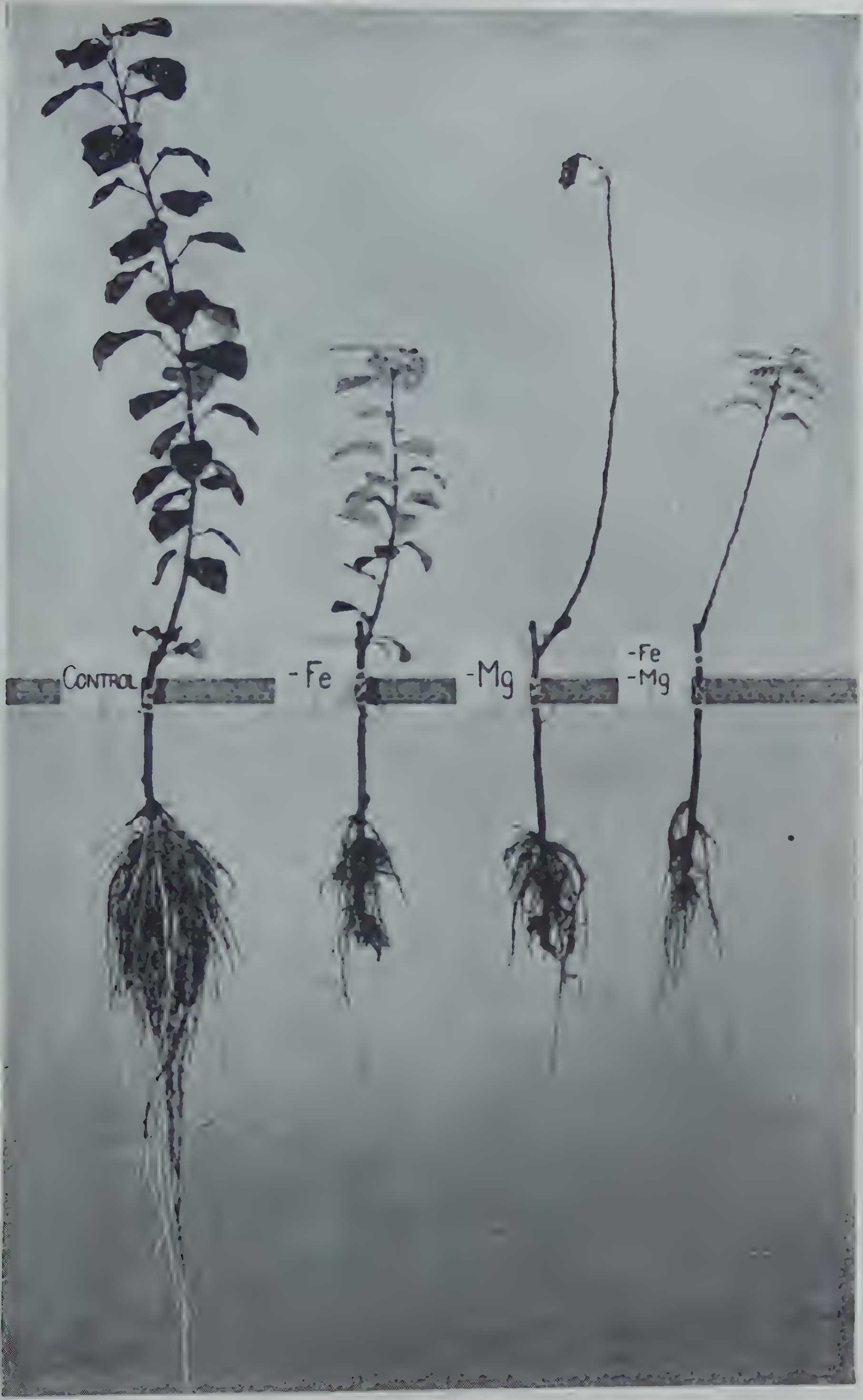


FIG. 1. Growth and symptoms on typical plants at the end of the season. Plant grown in (left to right): complete nutrient solution; low iron; low magnesium; low iron and low magnesium.

which developed into a light brown necrosis. Interveinal necrosis also appeared, often edged by a very thin band of paler green, or purple tissue, while the rest of the leaf remained dark green. Necrosis progressed until severely affected leaves were tattered and often curled downwards from the midrib; abscission followed. As the deficiency symptoms spread up the shoot, marginal or interveinal patches on the leaves nearer the apex suddenly lost turgor and turned necrotic within a few days. By the end of the season the defoliated stem bore only a tuft of apparently healthy leaves at the base and one or two leaves at the apex.

Combined iron and magnesium deficiency: The symptoms were similar to those already described for the separate deficiencies except that the chlorotic apical leaves caused by the iron deficiency did not readily become necrotic or absciss when the magnesium-deficiency symptoms reached them.

Fig. 1 shows a typical plant from each treatment exhibiting the characteristic form and symptoms at the end of the season.

Tables 1 and 2 give examples of the iron and magnesium concentration respectively, occurring in the leaves; each figure represents the mean of approximately nine determinations.

TABLE 1

Iron Content of Leaves, expressed as p.p.m. of Dry Weight

Date	Leaf group	Normal Fe Normal Mg	Low Fe Normal Mg
7 July . .	Middle	54	44
	Apical	58	35
18 August .	Middle	82	54
	Apical	69	42

TABLE 2

Magnesium Content of Leaves, expressed as a Percentage of Dry Weight

Date	Leaf group	Normal Fe Normal Mg	Normal Fe Low Mg
7 July . .	Middle	0·095	0·080
	Apical	0·099	0·088
18 August .	Middle	0·095	0·047
	Apical	0·107	0·071

Measured Group of Plants

Seasonal trends in growth for all treatments can be seen in Fig. 2*a*–*d*. The decrease in fresh weights and leaf areas of the magnesium-deficient plants after August can be accounted for by the severe leaf-fall.

Fresh weights (Fig. 2*a*): The weights were adjusted by means of analysis of covariance by those obtained on 14 June, i.e. before treatments were started. In comparison with the complete nutrient treatment, low iron in the presence of magnesium resulted in a low increment in total fresh weight by 9 August;

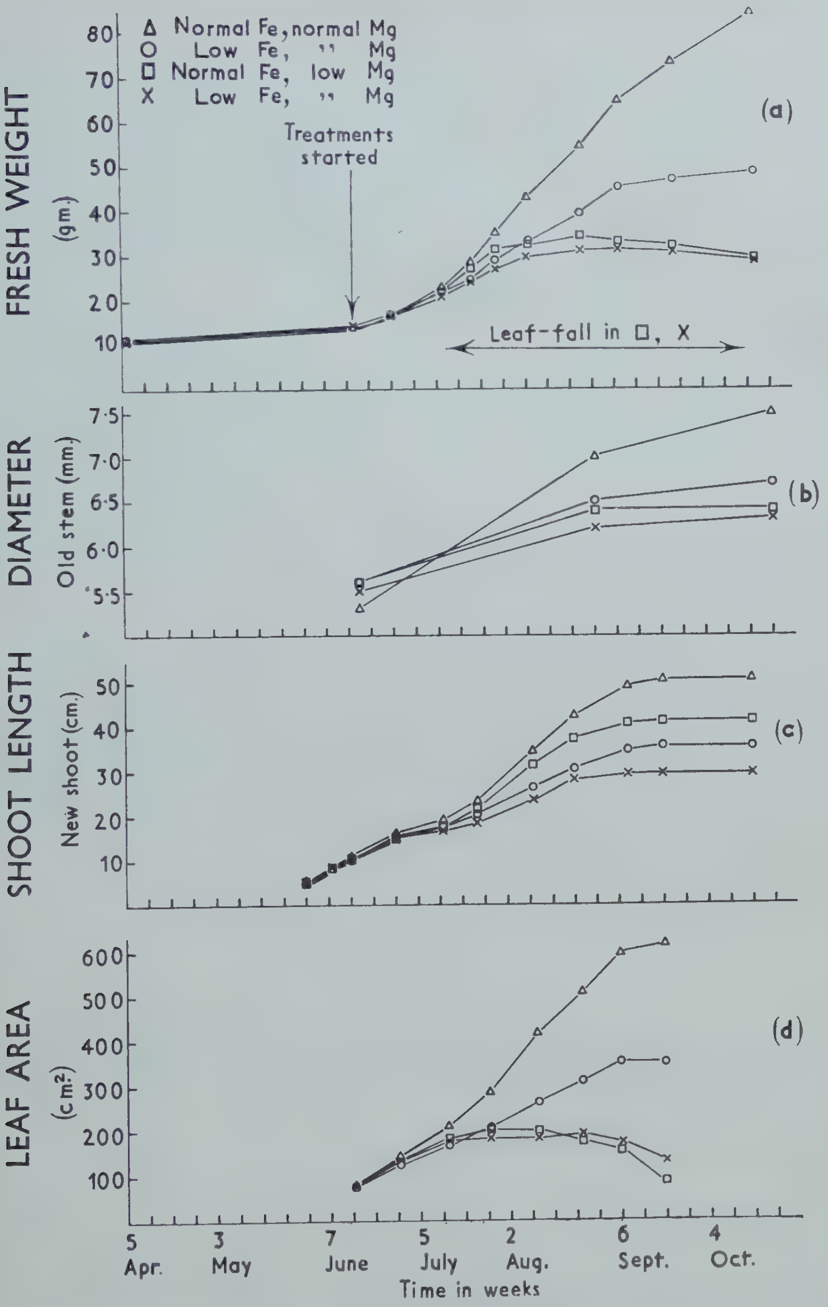


FIG. 2. Seasonal trends in growth: (*a*) total fresh weight per plant; (*b*) diameter of old stock; (*c*) new shoot length; (*d*) total leaf area per plant.
(*a–c*) mean of 36 plants per treatment; (*d*) mean of 24 plants per treatment.

by 20 October the fresh weight of the low-iron plants was only about half that of the controls.

It was difficult to make an accurate statistical assessment of the effect on fresh weight of omitting magnesium because, in August, the variance in the eight low-magnesium containers was not equal and, in October, the plants receiving magnesium were more variable than the deficient ones. (The coefficient of variation within containers was: Normal Mg = 18·1 per cent., Low Mg = 12·7 per cent.) In spite of this variability the differences between treatments were so great that they were significant at least at the 5 per cent. level.

Diameter of old stem (Fig. 2*b*): Deficiencies of iron and magnesium considerably reduced diameter increments, omission of magnesium having the greater effect. No significant further reduction was caused by the double deficiency. In both treatments receiving low magnesium thickening had ceased before the end of August. Statistical analyses were carried out on the increment of logarithms of the diameters; the actual diameters are shown in Fig. 2*b*.

New shoot length (Fig. 2*c*): Iron deficiency severely checked shoot extension growth while low magnesium had little effect until later in the season. There was no interaction between the two deficiencies.

Leaf areas (Fig. 2*d*): When iron was deficient leaf growth was reduced in two ways; not only were there fewer leaves formed but the chlorotic leaves were not as large as those of the same age on the control plants (Table 3). However, when the average leaf size per tree was plotted against its corresponding shoot length, there was a straight-line relationship which held both for the controls and for the iron-deficient plants at a given date. Thus although the leaves of iron-deficient plants were smaller than those of the controls they were, in fact, no smaller than would be expected from plants with such short shoots. There was little leaf-fall from the low-iron plants.

TABLE 3

Leaf Growth

	Date	Normal Fe Normal Mg	Low Fe Normal Mg	Normal Fe Low Mg	Low Fe Low Mg	Sig. diff. ($P = 0{\cdot}05$)
Total leaf area per plant in cm.^{2} .	11 August	416	261	200	187	48
	20 Sept.	616	350	83	132	102
Area per mature leaf in cm.^{2} .	11 August	13·9	9·5	—	—	1·8
No. of leaves developed per plant . .	20 Sept.	40·6	35·6	37·3	32·6	3·6

The main effect of low magnesium was severe leaf-fall which became progressively worse. From mid-August onwards this leaf-fall was removing a greater area of leaf than was expanding at the stem apex and the total leaf area remaining on the plant was actually being reduced. The number of leaves formed was not affected until later in the year at a time when shoot extension was also decreasing. It was impossible to assess the effect of magnesium deficiency on leaf size since many leaves rapidly became necrotic and abscissed before their full size had been measured, but they did not appear to differ in size from those of the control plants. This is in agreement with Mulder (1950) and Wallace (1930) who found that magnesium deficiency did not reduce the size of apple leaves in the first year.

At the end of the season there was a strong interaction between the effects of iron and magnesium deficiency when combined, since in this case more leaves remained on the shoots than when magnesium alone was low.

Harvested Group of Plants

Total dry weight per plant: Fig. 3 shows the mean accumulation of dry matter in the whole plant and its component parts as determined from the sample of four plants per treatment taken at each harvest. Apparent decreases in total dry weight, such as that found between harvest 4 and 5 in the double deficiency treatment, are obviously due to the variability of the plants. By October the total dry weight of plants grown with a low iron supply was only about 60 per cent. of that of the controls. Plants with low magnesium had only about 40 per cent. as much dry matter as the controls but leaf-fall, in part, accounted for this low figure. The relationship of total dry weight to total fresh weight was approximately linear so that the general trends of dry-weight accumulation were similar to those shown by the fresh weight curves of the measured plants (Fig. 2*a*). For the conversion of fresh weights of measured plants to dry weights, however, it was found necessary to use a separate regression line for each treatment, which remained valid throughout the season.

Dry weight of component parts: At the time of planting only the old stem was present so that, with this one exception, the total dry weight of each component is synonymous with its dry-weight increment from planting time. It can be seen from Fig. 3 that both iron and magnesium deficiencies led to a lower dry weight in all components.

The changing dry-weight relations of the different parts of the plant are best shown by expressing their dry-weight increments as percentages of the total increment in each treatment (Fig. 4*a*). Percentage dry-weight increments were also calculated excluding the leaves (Fig. 4*b*) because leaf-fall from the magnesium-deficient plants again complicated the issue and it was not possible to make any correction for this factor.

Both Figs. 4*a* and 4*b* show that all parts of the iron-deficient plants were reduced proportionally when compared with the controls. Magnesium-deficient plants had an abnormally low proportion of their dry matter present as roots and leaves, the latter low value being largely due to leaf-fall (Fig. 4*a*). In Fig. 4*b*, from which the leaf dry weights have been omitted, it is evident that, in low-magnesium treatments, the new shoot had the greater proportion of the available dry weight while the roots had a smaller and smaller proportion as the season advanced; the amount accumulating in the old stem was reduced. Camp (1947) also found that poor root systems were produced by magnesium-deficient citrus, but this is in contrast to the findings of Colby (1933) with French Prune, and Michael (1956?) with maize.

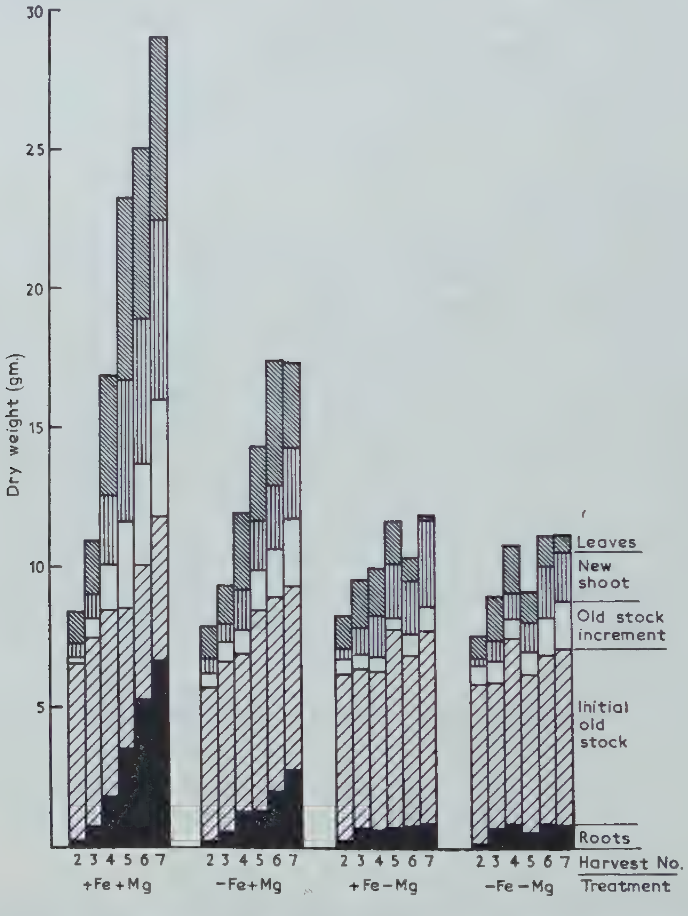


FIG. 3. Dry weights of component parts of plants throughout the season. Mean of 4 plants per treatment per harvest.

Harvest No.	Date	Harvest No.	Date
1	9–10 June	5	6–9 September
2	5–9 July	6	27–30 ,,
3	26–29 ,,	7	22 October
4	16–19 August		

In the double deficiency the new shoot accumulated considerably less of the available dry matter than it did in the magnesium-deficient plants in the presence of iron. On the other hand, the amount of available dry matter accumulating in the old stem of plants with the double deficiency was relatively more than that of either the controls or the plants deficient in magnesium alone. By comparing the ratios of root/total dry-weight increment of deficient plants with those of similar sized controls it was shown that the altered distribution of dry matter was a real effect of treatment and not due to the small size of the deficient plants.

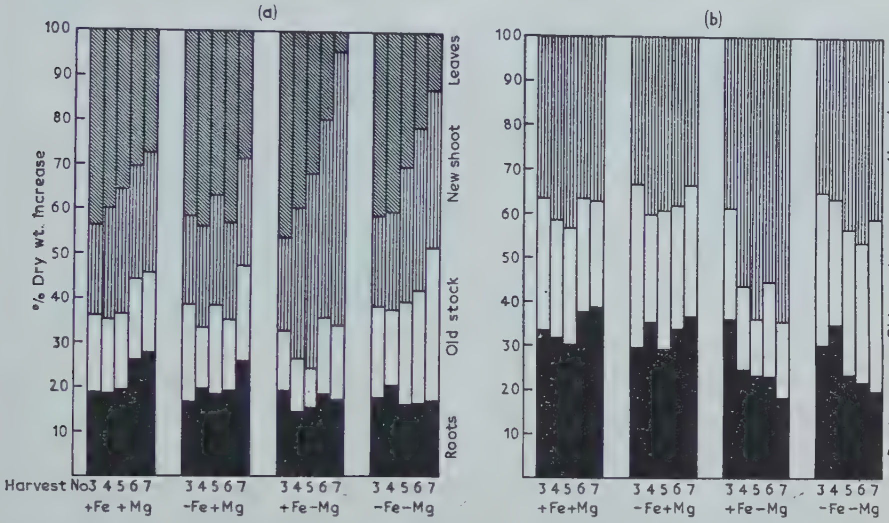


FIG. 4. Increase, from planting time, in dry weight of each component part of plant expressed as a percentage of the total increase per plant. Mean of 4 plants per treatment per harvest. Dates of harvests as in Fig. 3.

(*a*) Leaf weight included in total dry-weight increase.
(*b*) Leaf weight excluded from total dry-weight increase.

The considerable increase in the shoot/root ratio (dry-weight increment) of the magnesium-deficient plants shown in Table 4 also indicates the very poor growth of the roots of these plants.

TABLE 4

Shoot/Root Ratio (Dry-weight Increment)

Date	Normal Fe Normal Mg	Low Fe Normal Mg	Normal Fe Low Mg	Low Fe Low Mg	Sig. diff. ($P = 0·05$)
22 October . .	1·63	1·97	4·66	4·15	1·19

Dry weight and water content of leaves per unit area: The leaf fresh weight expressed on an area basis was similar in all treatments for leaves in similar positions on the shoots. If, however, the two constituents of fresh weight (dry matter and water content) were considered separately, effects due to iron deficiency were apparent (Fig. 5). The dry matter per square centimetre of leaf was lower while the water content was higher than that of similar leaves on the control plants, particularly in leaves on the middle of the shoot. This held throughout the season. Iljin (1952) also found a high water content associated

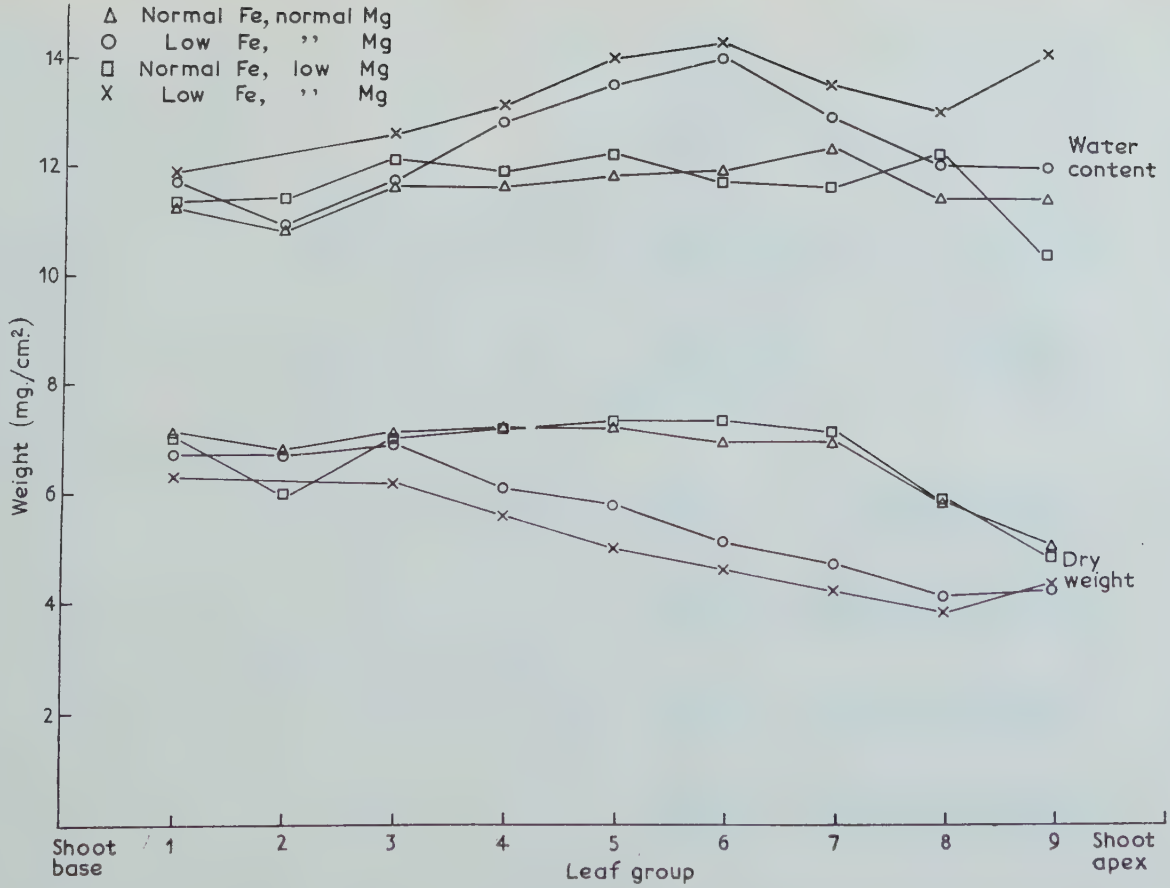


FIG. 5. Lamina dry weight and water content per unit area for different groups of leaves up the shoot at harvest 3 (26–29 July). Mean of 4 plants per treatment.

with chlorotic foliage in a number of species. However, as Richards and Shih (1940) have pointed out, the water relations of leaves are highly complex and dependent upon the external and internal concentrations of several elements.

Magnesium deficiency had no effect.

To show differences in the amount of dry matter per square centimetre of leaf and the way in which this varied with position on the shoot it was necessary to group the leaves in some way, while allowing for the fact that the shoots were growing at different rates and did not all carry the same number of leaves. In 1953 the leaves on a shoot were divided, according to position on the shoot, into five groups, all groups on any one shoot having the same number of leaves. Comparison of a group of leaves from the low-iron treatment with the corresponding group of control leaves showed no regular differences in dry weight and water content. In 1954, however, the youngest emergent leaf on every shoot was marked each week and when harvested the leaves were dealt with in these weekly groups of similar age. Treated in this way the dry weight and water content data showed consistent results as stated.

Relative Growth Rate

The six plants per container (twenty-four per treatment) whose leaf areas had been measured provided the data for these calculations.

Fresh weights were obtained at intervals of approximately a fortnight during the season (16 June to 25 September) and dry weights were derived by regression from the fresh weights as already described. The weights of leaves falling off during a fortnightly period were estimated from their areas and were added to the total dry weight of the plant at the end of that period. The relative growth rate (Blackman, 1919; Briggs, Kidd, and West, 1920*a*, 1920*b*) was calculated here as the increase in the natural logarithm of the dry weight per unit time.

In the control plants the maximum relative growth rate occurred at the end of July (Fig. 6), after which it fell throughout the season, apart from a small rise at the end of August. When compared with the controls both the low-iron and the low-magnesium treatments decreased the relative growth rate throughout the season. The reduction in growth rate due to the double deficiency was only slightly more than that caused by each single deficiency.

Each of the figures in Table 5 represents the mean of three consecutive values of the relative growth rate.

Net Assimilation Rate

The net assimilation rate (Briggs, Kidd, and West, 1920*b*; Gregory, 1926) was calculated here as the increment in dry weight per unit time, divided by the arithmetic mean leaf area. Over these short periods of time very little difference was found between results from this formula and from that which assumes leaf expansion to be non-linear. The data were the same as those used for the relative growth rate calculations with the addition of leaf area measurements made on the same plants.

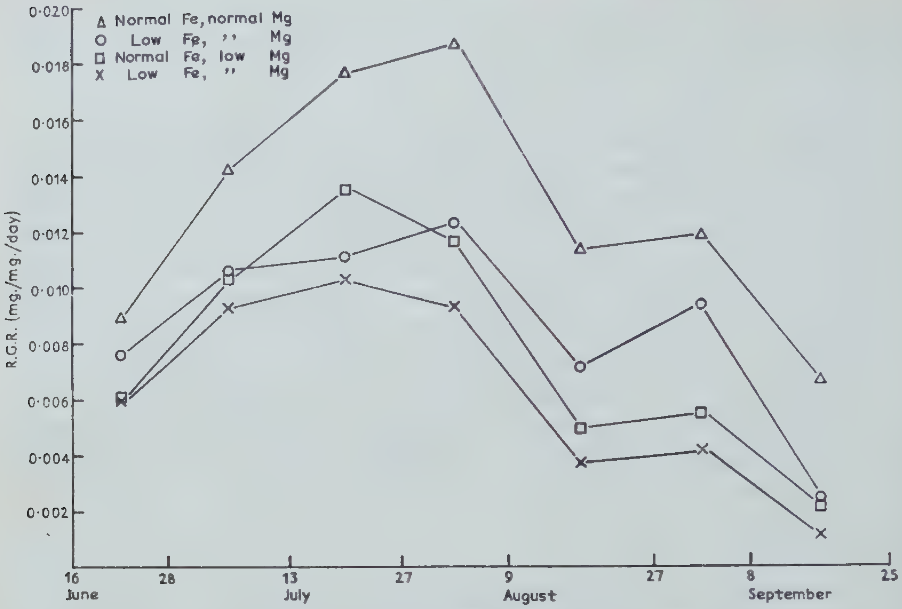


Fig. 6. Seasonal trends in relative growth rates; for clarity the rate over a period is represented as a point inserted at the middle of that period. Mean of 24 plants per treatment.

Table 5

Relative Growth Rates, Net Assimilation Rates, and Leaf Area Ratios

	Date	Normal Fe Normal Mg	Low Fe Normal Mg	Normal Fe Low Mg	Low Fe Low Mg	Sig diff. ($P = 0{\cdot}05$)
R.G.R. . . (mg./mg./day)	28 June to 9 August	0·0168	0·0113	0·0118	0·0096	0·0018
	9 August to 25 Sept.	0·0099	0·0062	0·0041	0·0030	
N.A.R. . . (mg./cm.^{2}/day)	28 June to 9 August	0·688	0·535	0·597	0·486	0·094
	9 August to 25 Sept.	0·341	0·254	0·297	0·167	
L.A.R. . . (cm.^{2}/mg.)	28 June to 9 August	0·0245	0·0215	0·0197	0·0200	0·0018
	9 August to 25 Sept.	0·0288	0·0245	0·0136	0·0170	

The leaf area and dry weight of the magnesium-deficient plants had to be corrected for necrosis and defoliation. Very different values for the net assimilation rate were obtained according to the corrections used. It might be argued that a leaf which became totally necrotic, or abscissed in the fortnight, would not have contributed appreciably to the dry weight in that period and therefore its area should not be included at all. This assumption, however, led to an increase in weight in the apparent absence of leaves and hence to an infinite value for the net assimilation rate, as in the case of some plants, in the last stages of the deficiency, whose leaves all became totally necrotic or abscissed during the period.

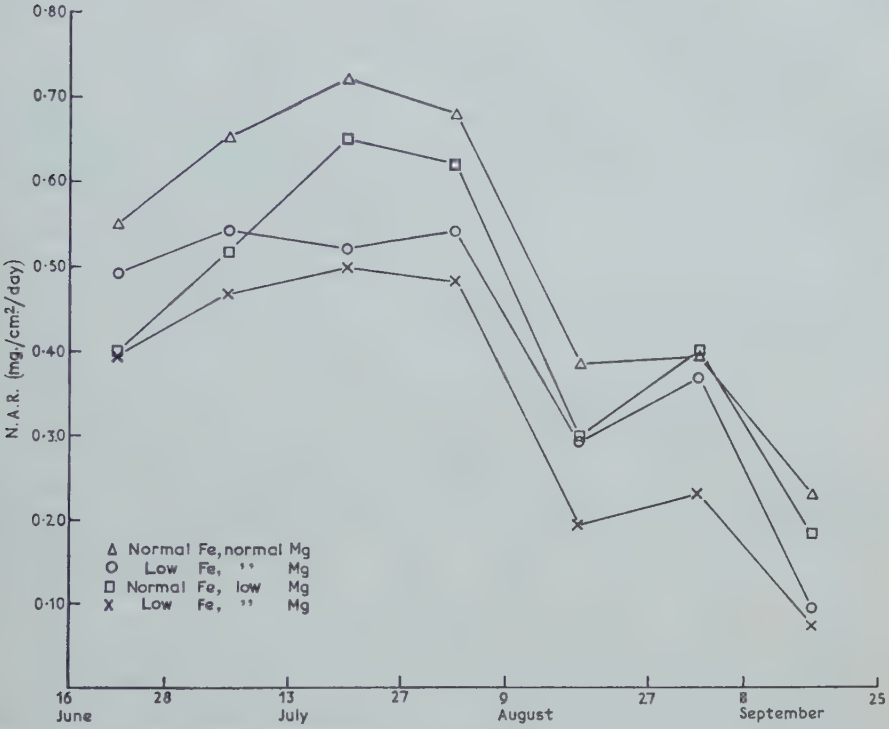


FIG. 7. Seasonal trends in net assimilation rates; the rate over a period is represented as a point as in Fig. 6. Mean of 24 plants per treatment.

The following procedure was adopted in an attempt to meet the difficulties of estimating the functional green leaf area under these conditions. Any necrotic parts were omitted from leaf area measurements; when a leaf abscissed before the end of a period then half its green area at the beginning was taken as its mean leaf area. The dry weight of such a leaf (estimated from its total area) was included in the total weight of the plant at both the beginning and end of the period.

The maximum net assimilation rate occurred about mid-July and was approximately 0·70 mg./cm.^{2}/day for the control treatment and 0·50 and 0·65 mg./cm.^{2}/day in the low-iron and low-magnesium treatments respectively (Fig. 7). Subsequently the net assimilation rate fell until the end of the season except for a slight rise in all treatments at the end of August similar to that found in the relative growth rates. In comparison with plants grown in complete nutrient solution, either low iron or low magnesium reduced the value over the season as a whole ($P < 0{\cdot}001$ in each case) and there was no significant interaction, the value for the combined deficiency being lower than either deficiency alone. The arithmetic means of the net assimilation rates for three fortnightly periods are given in Table 5 from which it can be seen that, over these short periods, the effect of magnesium deficiency is less than that of iron deficiency.

Leaf Area Ratio

Estimates of the leaf area ratios in cm.^{2} of leaf per mg. of dry weight of plant were obtained by dividing the relative growth rates by the corresponding net assimilation rates using the treatment means, and were not calculated directly from the original data (Briggs, Kidd, and West, 1920*b*).

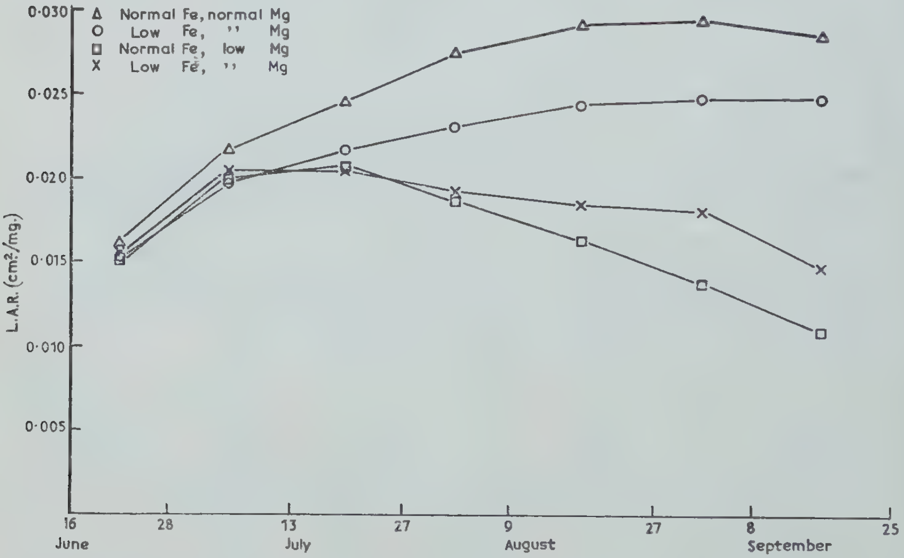


FIG. 8. Seasonal trends in leaf area ratios; calculated from relative growth-rates and net assimilation rates. The rate over a period is represented as a point as in Fig. 6.

In the control plants the leaf area ratio increased throughout the season until the last period (Fig. 8). In all three deficient treatments the ratio was lower than that for the control. Whereas the ratio for iron-deficient plants in the presence of magnesium rose steadily throughout the season (although more slowly than that for the control), the ratio for the plants grown with low magnesium dropped steadily after July, owing to the considerable leaf-fall. Since there was less leaf-fall in plants with the double deficiency the leaf area ratio was not as low in this treatment as that for plants with magnesium deficiency in the presence of iron.

DISCUSSION

Plants grown with solutions of low iron content showed a low relative growth rate compared with the controls, as did those grown with a solution of low magnesium content, but the plants of these two deficient treatments showed morphological and physiological differences. In the low-iron plants the distribution of new dry matter in the different parts of the plant was approximately the same as in the controls, while in the low-magnesium plants the development of the various parts showed marked differential effects.

The leaves of the iron-deficient plants, apart from the first few leaves formed, were chlorotic, but it is not possible to state whether or not their rate of photosynthesis was low, in the absence of direct measurements of either respiration or photosynthesis. The ratio of dry-weight increment of leaf to dry-weight increment of plant was the same in the low-iron as in the control plants and this ratio showed the same seasonal change in both treatments. The leaves of the low-iron plants, however, had a lower dry weight per unit area so that the total leaf area was high in relation to plant dry-weight increment, and this resulted in low values for net assimilation rate. On the other hand, because the plant dry-weight increment was relatively small in proportion to the contribution of the initial old stem to the total weight, the dry weight and the area of the leaves of the low-iron plants were always low in relation to the total plant dry weight, i.e. the leaf area ratio was low. This higher proportion of respiring tissue in the low-iron plants would itself tend to lower the values of both net assimilation rate and relative growth rate, irrespective of any real change in plant respiration rate per unit dry matter or in photosynthetic efficiency of the leaves.

Plants in all treatments came originally from the same population, but the iron-deficient plants grew less than the controls when treatments were applied. Three possibilities, either singly or in combination, suggest themselves as causes of this initial reduction in growth. (1) The chlorotic leaves might have a lower photosynthetic efficiency; (2) the respiration rate of the plants might have increased; (3) the proportion of dry matter accumulating in the various parts of the plant might have altered resulting in a decrease in the ratio of photosynthetic to respiring tissue. The third possibility is unlikely since the percentage increments of dry matter in the leaves, shoots, and roots were similar in both the iron-deficient and the control plants throughout the season. The initial decrease in growth-rate is therefore likely to have been due to either or both of the first two causes. Glenister (1944) and Somers, Gilbert, and Shrive (1942) found a decrease, not an increase, in respiration in sunflower and soybean with a low iron supply and, if this holds here, then a decrease in photosynthesis remains as the possible cause of reduced growth. However, such a reduction in photosynthetic rate need not necessarily be due to any inherent inefficiency in the photosynthetic mechanism. Thus, Sideris and Young (1956) found that in iron-deficient pineapple, although chlorophyll synthesis was very low, both sugar and starch were produced in ample amounts and their concentration at times even exceeded that in the controls. This suggests that they were not being utilized and that reduced meristematic activity was causing accumulation of carbohydrates which in turn might have reduced photosynthesis. Once the difference in size between the low-iron and control plants had been established, the leaf area ratio of the former would be reduced as explained in the preceding paragraph and this would then lead to a reduction in relative growth rate and net assimilation rate.

The foliar symptoms and the morphology of the magnesium-deficient plants were in contrast to those in plants suffering from iron deficiency; chlorosis was absent and there was severe necrosis and leaf-fall. The type of symptom occurring on the older leaves was normal, but the flaccid areas which appeared suddenly on the younger leaves were not so typical and are of interest since Mulder (1950) has stated that leaves with magnesium deficiency showed increased transpiration.

Owing to the severe necrosis and progressive leaf-fall, the leaf area ratio of the magnesium-deficient plants fell steadily for most of the season and thus the proportion of respiring tissue to leaf area rose. It is probable that this was one of the chief causes of the reduction in relative growth rate and net assimilation rate, rather than any effect of the treatment upon photosynthetic efficiency or on respiration rate. The net assimilation rate was slightly lower throughout the season than that of the control plants. Neales (1955) found that the net assimilation rate was markedly reduced in magnesium-deficient barley; this plant, unlike M.VII rootstock, becomes chlorotic when deficient in magnesium.

The new shoot in the low-magnesium plants retained a larger proportion of the available assimilates, while the old stem and even more the roots accumulated relatively little. Magnesium deficiency therefore led to a small root system and a comparatively large top. Further, comparison of the dry weights of the new shoots in relation to their lengths shows that their form was altered in that the reduction in length was slight compared with the reduction in diameter. This suggests that the activity of the apical meristem was affected less than that of the cambium.

Among the interactions noted between iron and magnesium deficiencies two were outstanding. The double deficiency caused a greater retention of leaves at the end of the season than when magnesium alone was low. Also, when both iron and magnesium were omitted, more material accumulated in the old stem and less in the new shoot than would be expected from a simple combination of the single deficiencies, suggesting increased translocation. However, since nothing is known of the respiration rates of the different parts of the plants, accumulation of assimilates does not necessarily reflect the amount of translocation taking place.

Some indications of the probable movements of iron and magnesium within the plants were obtained from observations of symptoms and of the development of the plants. Thus the first leaves formed on plants grown in low-iron solution remained green while the leaves higher up the shoot were extremely chlorotic, confirming the general impression that iron, once taken up by the leaves, does not move freely to other parts of the plant. The primordia of these first few leaves had been present in the dormant bud since the preceding season and may then have been able to accumulate enough iron to meet their needs for chlorophyll synthesis.

Rogers, Batjer, and Thompson (1953) give evidence that magnesium does not move from apple leaves, since the magnesium content remained constant in the same leaves throughout the season when calculated on an area basis, but the work of Reed and Haas (1924), Fudge (1939), Phillis and Mason (1942), and Cain (1953) suggests that magnesium is mobile. The results set out in this paper support the view that magnesium was free to move from the leaves near the base, where necrosis developed, to the apex, where newly formed leaves were green. This movement of magnesium to the apex is further suggested by the small effect of magnesium deficiency on the activity of the apical meristem compared with the marked adverse effect on secondary thickening. These are, however, merely indications, confirmation of which will depend on examination of the chemical data.

SUMMARY

1. One-year rooted shoots from stoolbeds of M.VII rootstocks were grown in a spray-culture apparatus for one season. After a preliminary period, during which all plants received the same solution, they were selected for uniformity and treatments were applied.

2. Treatments consisted of (*a*) a complete nutrient solution, (*b*) nutrient solution low in iron, (*c*) nutrient solution low in magnesium, and (*d*) nutrient solution low in both iron and magnesium.

3. In spite of reserves present in the old stem, symptoms of iron and magnesium deficiencies first appeared 11 and 12 weeks respectively after 'planting'. Iron deficiency caused chlorosis with hardly any necrosis or defoliation. With magnesium deficiency there was very little chlorosis but severe necrosis developed progressively up the shoot, followed by premature leaf-fall. Plants receiving low iron and low magnesium showed both sets of symptoms, but the necrosis and abscission did not progress as far up the shoot as when magnesium alone was low.

4. Total leaf areas, area per leaf, and number of leaves formed on plants low in iron were all reduced when compared with the controls, but were in fact no smaller than would be expected from such small plants with short shoots. The water content of iron-deficient leaves was increased, whether it was based on area or on dry weight. Owing to rapid defoliation it is impossible to state accurately the size of magnesium-deficient leaves, but evidence suggests they were no smaller than those of the controls. Later in the season the number of leaves produced was lower than in the controls, as the elongation of the new shoot slowed down. More leaves remained at the end of the season on plants low in both iron and magnesium than on those with magnesium deficiency alone.

5. The relative growth rates of plants in all deficiency treatments were lower than the controls, but there were morphological and physiological differences between plants grown with low iron and those grown with low magnesium.

6. In the low-iron plants there was an approximately proportional reduction in dry-weight increments of all parts of the plant measured, as compared with the controls. In the magnesium-deficient plants, more of the available dry matter accumulated in the new shoot, less in the old stem, and very little in the roots. The reduction in length of the new shoot was slight compared with the reduction in diameter. Plants deficient in both iron and magnesium accumulated more dry matter in the old stem and less in the new shoot than would have occurred with a simple combination of the single deficiencies.

7. The shoot/root ratios were similar in the low-iron plants and the controls, but were increased in all plants low in magnesium because of the very poor root systems developed.

8. Compared with the controls the net assimilation rates were consistently lower throughout the season for the magnesium-deficient plants and further reduced for plants with low iron or the combined deficiency. The leaf area ratios were also decreased in the three deficiency treatments, the greatest effect being on the low-magnesium plants and the least effect on those with low iron.

The relation of these to the relative growth rates and morphology of the plants is discussed.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. D. W. P. Greenham and Dr. B. D. Bolas for constant advice in all matters concerning the preparation of this paper, Dr. W. A. Roach who initiated the work and developed the spray culture method, and Mr. R. Neve who helped to build and maintain it. They would also like to acknowledge the help given by Mr. A. C. Mason and his staff for the chemical analyses and for suggesting suitable nutrient solutions, also Mr. G. H. Freeman and members of the Statistics Section for advice and for carrying out many of the statistical analyses, and Mr. J. Elliott for summarizing some of the data.

LITERATURE CITED

BLACKMAN, G. E., and RUTTER, A. J., 1948: Physiological and Ecological Studies in the Analysis of Plant Environment. 3. The Interaction between Light Intensity and Mineral Nutrient Supply in Leaf Development and in the Net Assimilation Rate of the Bluebell (*Scilla nonscripta*). Ann. Bot., N.S., Lond., **12**, 1–26.

BLACKMAN, V. H., 1919: The Compound Interest Law and Plant Growth. Ann. Bot., Lond., **33**, 353–60.

BRIGGS, G. E., KIDD, E., and WEST, C., 1920*a*: A Quantitative Analysis of Plant Growth. Part 1. Ann. appl. Biol. **7**, 103–23.

—— —— —— 1920*b*: A Quantitative Analysis of Plant Growth. Part 2. Ibid. 202-23.

BROWN, J. C., 1956: Iron Chlorosis. Annu. Rev. Pl. Physiol., **7**, 171–90.

CAIN, J. C., 1953: The Effect of Nitrogen and Potassium Fertilizers on the Performance and Mineral Composition of Apple Trees. Proc. Amer. Soc. hort. Sci., **42**, 46–52.

CAMP, A. F., 1947: Magnesium in Citrus Fertilization in Florida. Soil Sci., **43**, 43–52.

COLBY, H. L., 1933: Seasonal Absorption of Nutrient Salts by the French Prune Grown in Solution Cultures. Plant Physiol., **8,** 1–34.

FUDGE, B. R., 1939: Relation of Magnesium Deficiency in Grapefruit Leaves to Yield and Chemical Composition of Fruit. Bull. Fla. agric. Exp. Sta., No. 331.

GLENISTER, P. R., 1944: Effects of Iron Deficiency on Respiration of Sunflower Plants. Bot. Gaz., **106,** 33–40.

GREGORY, F. G., 1926: The Effect of Climatic Conditions on the Growth of Barley. Ann. Bot., Lond., **40,** 1–26.

—— and RICHARDS, F. J., 1929: Physiological Studies in Plant Nutrition. 1. The Effect of Manurial Deficiency on the Respiration and Assimilation Rate in Barley. Ibid., **43,** 119–61.

ILJIN, W. S., 1952: Metabolism of Plants Affected with Lime-induced Chlorosis (Calciose). 3. Mineral Elements. Plant and Soil, **4,** 11–28.

KRACKENBERGER, H. F., and PETERSON, W. J., 1954: Effect of Environment on the Magnesium Content of Plants. Southern Co-operative Series Bull., **36,** 98–118.

MASON, A. C., 1953: The Cleaning of Leaves Prior to Analysis. Rep. E. Malling Res. Sta. for 1952. 104–7.

MICHAEL, G., (1956?): The Absorption and Distribution of Magnesium and its Function in the more Complex Green Plant. Magnesium—a Plant Food. Bergbau–Handel. (Translation from the German edition.) 131–222.

MORTON, A. G., and WATSON, D. J., 1948: A Physiological Study of Leaf Growth. Ann. Bot., N.S., Lond., **12,** 281–310.

MULDER, D., 1950: Magnesium Deficiency in Fruit Trees on Sandy Soils and Clay Soils in Holland. Plant and Soil, **2,** 145–57.

NEALES, T. F., 1955: Relation of Magnesium Supply to Growth and Net Assimilation Rate in Barley. Nature, Lond., **175,** 429–30.

PHILLIS, E., and MASON, T. G., 1942: On Diurnal Variations in the Mineral Content of the Leaf of the Cotton Plant. Ann. Bot., N.S., Lond., **6,** 437–42.

REED, H. S., and HAAS, A. R. C., 1924: Nutrient and Toxic Effects of certain Ions on Citrus and Walnut Trees with Especial Reference to the Concentration and pH of the Medium. Tech. Pap. Calif. agric. Exp. Sta., No. 17. 1–75.

RICHARDS, F. J., and SHIH, S., 1940: Physiological Studies in Plant Nutrition. 10. Water Content of Barley Leaves as Determined by the Interaction of Potassium with Certain other Nutrient Elements. Part 1. The Relationship between Water Content and Nutrient Composition. Ann. Bot., N.S., Lond., **4,** 165–75.

ROACH, W. A., NEVE, R., VANSTONE, F. H., PHILCOX, H. J., DELAP, ANNE V., and FORD, ELSIE M., 1957: A Method of Growing Apple Trees by Spraying their Roots with Nutrient Solution. J. hort. Sci., **32,** 85–98.

ROGERS, B. L., BATJER, L. P., and THOMPSON, A. H., 1953: Seasonal Trend of Several Nutrient Elements in Delicious Apple Leaves expressed on a per cent. and Unit Area Basis. Proc. Amer. Soc. hort. Sci., **61,** 1–5.

RUCK, H. C., and BOLAS, B. D., 1954: The Effect of Manganese on the Assimilation and Respiration Rate of Isolated Rooted Leaves. Ann. Bot., N.S., Lond., **18,** 267–97.

SIDERIS, C. P., and YOUNG, H. Y., 1956: Pineapple Chlorosis in Relation to Iron and Nitrogen. Plant Physiol., **31,** 211–22.

SMITH, P. F., REUTHER, W., and SCUDDER, G. K., 1953: Effect of Differential Supplies of Nitrogen, Potassium and Magnesium on Growth and Fruiting of Young Valencia Orange Trees in Sand Culture. Proc. Amer. Soc. hort. Sci., **61,** 38–48.

SOMERS, I. I., GILBERT, S. C., and SHRIVE, J. W., 1942: The Iron-manganese Ratio in Relation to the Respiratory CO_2 and Deficiency-toxicity Symptoms in Soybeans. Plant Physiol., **17,** 317–20.

SPEIRS, MARY, 1954: Effect of Environment on the Iron Content of Plants. Southern Co-operative Series Bull., **36,** 155–75.

WALLACE, T., 1930: Experiments on the Manuring of Fruit Trees. 3. The Effects of Deficiencies of Potassium, Calcium, and Magnesium, respectively, on the Contents of these Elements, and of Phosphorus in the Shoot and Trunk Regions of Apple Trees. J. Pomol., **8,** 23–43.

—— 1939: Magnesium-deficiency of Fruit Trees. Ibid., **17,** 150–66.

—— 1940: Chemical Investigations relating to Magnesium Deficiency of Fruit Trees. Ibid., **18,** 145–60.

WALLACE, T., 1951: Mineral Deficiencies in Plants. 2nd edition. H.M.S.O., Lond. 107 pp.
WATSON, D. J., 1947: Comparative Physiological Studies on the Growth of Field Crops. 2. The Effect of Varying Nutrient Supply on Net Assimilation Rate and Leaf Area. Ann. Bot., N.S., Lond., **11**, 375–407.
WEINSTEIN, L. H., MEISS, A. N., UHLER, R. L., and PURVIS, E. R., 1956: Growth-promoting Effects of Ethylenediamine Tetra-acetic Acid. Nature, Lond., **178**, 1188.
ZIMMERMAN, MIRYAM, 1947: Magnesium in Plants. Soil Sci., **63**, 1–12.

Observations of the Effect of Water on the Hyphal Apices of *Fusarium oxysporum*

BY

N. F. ROBERTSON

(*Botany School, University of Cambridge*)

With four Figures in the Text

Abstract

When colonies of *Fusarium oxysporum*, growing on plates of mineral-sucrose agar, are flooded with the mineral-sucrose solution, without added agar, or with solutions of any of the constituents of the mineral-sucrose mixture at a concentration of 0·076 M the leading hyphal apices at the agar surface continue to grow on unchecked. If, however, the colonies are flooded with solutions of decreasing and increasing molarity from 0·076 M an increasing proportion of the leading hyphal apices at the agar surface stop growing, and branch subterminally. In distilled water about 50 per cent. of the apices branch and this branching is preceded by swelling, whereas in 0·5 M sucrose more than 90 per cent. of the apices branch and the branching is not accompanied by swelling. In the distilled water those hyphae which do not branch swell a little and grow on from the apex within 40 seconds.

When hyphal apices are flooded with distilled water for from 10 to 40 seconds and then transferred to mineral-sucrose solution more than 90 per cent. of the hyphal apices branch, whereas flooding with distilled water for 60 seconds or longer gives the same percentage of branched apices as does flooding with distilled water alone.

It is shown that swelling and branching of the hyphal apex are not causally related but that branching always occurs following arrestment of the hyphal apex for more than 60 seconds. It is suggested that the phenomena reported can be explained in terms of an irreversible change in the apical cap of the arrested hypha such that continued extension can no longer take place in this region and fresh outlets for growth must then be found subterminally. Such a mechanism, however triggered, could account for a wide variety of morphogenetic forms in the fungi.

Introduction

In attempts to determine the osmotic pressure of the vacuolar sap of the phialides of *Fusarium oxysporum* it was observed that the solutions used to flood the colonies on agar plates had profound effects on the morphology of the apices of the main growing hyphae. The morphogenetic effects were so striking that it was of interest to analyse the mechanism of their production. In the course of the experimental work it became clear that the simple techniques used could provide information of value for an understanding of the mechanism of growth of the hyphal apex and of its water relations. No general picture of the growth of hyphae can yet be given but because of the possible applications of the technique it seems appropriate to give in some detail the analysis already made.

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

MATERIAL AND METHODS

The fungus used in the investigation was *F. oxysporum* (Schlecht.) Fr. f. *gladioli* Snyder and Hansen. A single spore isolate was derived from isolate 87 of Buxton (1955) and has also been used in experimental studies by Carlile (1956). In the beginning cultures were made by streaking one loopful of a standard microspore suspension across the centre of a plate of mineral sucrose agar of formula (per cent. w.v.): agar, 1·5; sucrose, 1·5; $NaNO_3$, 0·2; KCl, 0·05; KH_2PO_4, 0·1; $MgSO_4$, $7H_2O$, 0·05; $FeSO_4$, trace. The cultures were grown at 22° C. in the dark for 60 hours before use. In later experiments inoculum was provided as no. 3 cork borings from the edge of a 3-day culture, the resulting colonies being used after 48 hours. Experiments were performed in the light at a temperature of *c.* 19° C., observations being made with the 1/3 objective of the microscope on the open, flooded plate. More detailed observations were made under cover-slips with the 1/6 and oil-immersion objectives. Quantitative results were obtained by recording the behaviour of a given number of consecutive hyphal apices; the position in the colony was selected at random and only the leading apices actually in focus at the agar surface were considered. Replication was obtained by counting the hyphal apices of colonies in different Petri-dishes, although sometimes two readings were made on one plate by counting the apices at opposite ends and on opposite sides of the elongated colony.

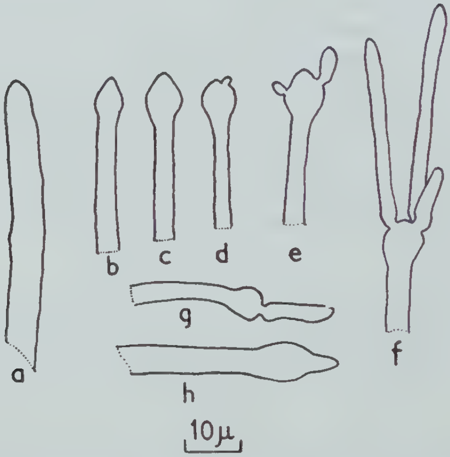


FIG. 1. Hyphal apices of *Fusarium oxysporum* at the surface of an agar plate. (*a*) Normal-growing hyphal apex. (*b*) Hyphal apex after *c.* 40 seconds in distilled water. (*c*), (*d*), (*e*), and (*f*) Hyphal apices showing progressive stages of swelling and branching in distilled water. (*g*) A hyphal apex showing regrowth by means of one branch. (*h*) A hyphal apex which has not continued to swell showing regrowth from the extreme apex.

OBSERVATIONS

1. *In water*

That part of the hypha from the last-formed septum to the extending apex consists of a wall and a cytoplasmic interior. As far as can be seen using ordinary light microscopy, the cytoplasm shows neither vacuoles nor obvious protoplasmic streaming. The apex is continually extending at a rate of about 5 μ per minute. When the colony is flooded with distilled water the extension immediately ceases. Thereafter the series of changes depicted in Fig. 1 takes place. In between 30 per cent. and 50 per cent. of the hyphae (exceptionally more than 60 per cent., see Table 4) the swelling of the hyphal apex, which is not accompanied by vacuolization, is followed after less than 1 minute by continued growth of the apex. As growth continues it is difficult to observe that these hyphae have been affected and they are recorded in the tables as 'unbranched'. In the remaining hyphae the swelling is a little greater in volume and is prolonged for 5–7 minutes before regrowth takes places. In this case the growth generally takes the form of two or more small protoplasmic protuberances on the shoulders of the swollen apex, which grow on as normal hyphae. A few apices produce only one such protuberance and the hypha grows on with an obvious kink to mark the position of arrestment, a few swell a great deal and do not grow on, and a few burst. Where two or more protuberances are formed on the swollen apex they may be formed simultaneously, but more usually one appears before the others (Table 1).

TABLE 1

Order of Appearance of Protuberances from Swollen Hyphal Apices in Distilled water

Colonies 60 hours on mineral-sucrose agar. One hundred apices recorded.

	With branching simultaneous	With left-hand branch first	With right-hand branch first
No. of instances	18	40	42

The new branches developing on plates flooded with distilled water will repeat the same sequence of events when the distilled water first used is replaced by fresh distilled water. If colonies of the fungus are allowed to spread on to glass coverslips, the hyphal apices react in a similar fashion to those on agar although fewer apices give rise to branches. A hyphal apex from a colony on a coverslip will also react to distilled water by swelling and branching when it is severed from the mycelium just behind the first septum.

2. *In sucrose solutions $> 0{\cdot}5$ M*

In all solutions of sucrose investigated, from 0·5 to 6 M, the immediate effect of flooding of an agar-plate colony was to stop the forward extension of the hyphae. In the solutions of lower concentration recovery took place relatively rapidly and a high proportion of the recovered apices showed two or more branches; in the higher concentrations, the hyphal apices showed shrinkage of the cytoplasm from the wall, recovery took longer, and a high proportion of the recovered tips showed only one branch. The results of these experiments are presented graphically in Fig. 2.

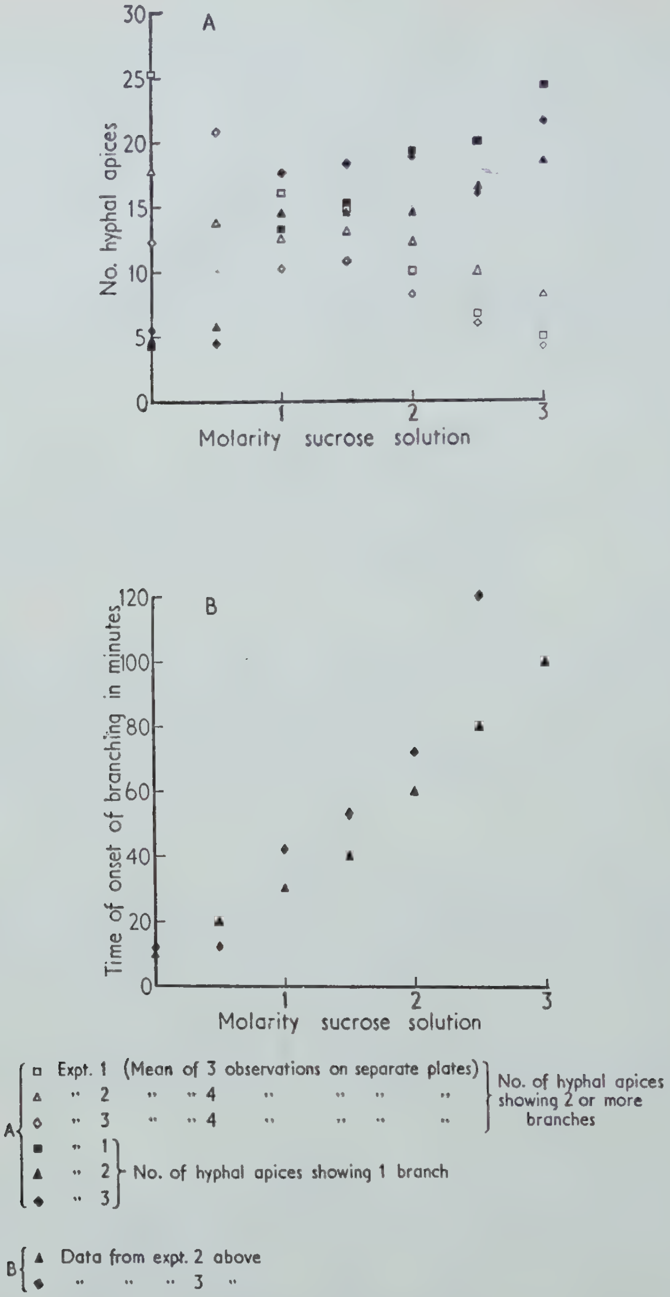


FIG. 2. A. The relation between molarity of sucrose solutions, in which the hyphae are immersed, and the numbers of hyphae showing one subterminal branch and two or more subterminal branches.

B. The relation between the molarity of the bathing solutions of sucrose and the time of onset of branching of the hyphal apex.

3. *In mineral-sucrose solution*

When the effect of the mineral-sucrose solution (already defined), without added agar, was investigated, the hyphal apices immersed in this solution did not show an appreciable amount of rebranching, and microscopic observations showed that their forward extension continued without a check (Table 2).

TABLE 2

Number of Hyphal Apices showing Branching on Treatment with Distilled Water or Mineral-sucrose Solution

Thirty consecutive apices counted in each of two dishes

Treatment		Hyphal apices unbranched	Hyphal apices one branch	Hyphal apices two branches
Distilled water	Dish 1	14	4	12
	Dish 2	11	4	15
Mineral-sucrose solution	Dish 1	26	2	2
	Dish 2	26	1	3

The effects of the mineral-sucrose solution with each of the constituents removed in turn and of sodium nitrate and sucrose separately were then tested (Table 3).

TABLE 3

Effect of Mineral-sucrose Solution, with and without each of its Constituents, on the Hyphal Apices of F. oxysporum

Thirty hyphae on each of three plates scored

Solution	Molarity	No. apices unbranched	No. apices with one branch	No. apices with two branches
Mineral-sucrose (M S)	0·0759	29	—	1
M S—$NaNO_3$	0·0319	28·66	—	1·33
M S—KH_2PO_4	0·0692	30	—	1
M S—KCl	0·0699	28·33	—	1·66
M S—$MgSO_4, 7H_2O$	0·0740	28·66	—	1·33
M S—Sucrose	0·0319	27·66	—	2·33
M S—($NaNO_3$+ KH_2PO_4)	0·0522	25·66	—	4·33
M S—(KCl+ $MgSO_4, 7H_2O$)	0·0680	29·33	—	0·66
M S—($NaNO_3$+ sucrose)	0·0089	20	—	10
$NaNO_3$ alone	0·0230	17·33	2·33	10·33
Sucrose alone	0·0440	25·33	0·33	4·33
Distilled water	0·0	15·33	2·0	12·66

Extension of the hyphal apices continued unchanged in all but two of the solutions, the mineral-sucrose solution without sodium nitrate and sucrose, and the nitrate solution alone. Both solutions differ from the others and agree with one another in having a low molar concentration of dissolved solutes.

This suggests that the difference between water and mineral-sucrose solution lies in the osmotic pressure of the latter and not in the proportion of dissolved salts.

To test this hypothesis an experiment was carried out in which the separate constituents of the mineral-sucrose solution were dissolved in distilled water to give a concentration of 0·076 M (the total molarity of the mineral-sucrose solution) and applied to the hyphal apices of the fungus (Table 4).

TABLE 4

Effect of 0·076 M Solutions of the separate Constituents of mineral-sucrose Solution on the Hyphal Apices of F. oxysporum*

Mean of four observations on samples of thirty hyphal apices

Solution	$NaNO_3$	KH_2PO_4	KCl	$MgSO_4$	Sucrose	Sucrose 0·152 M	Water
No. apices unbranched . .	28	26·5	29·25	26·25	28·25	29·25	21·25
No. apices with one branch . .	0·25	0·5	0·25	0·25	0	0	0
No. apices with two or more branches	1·75	3·0	0·5	3·5	1·75	0·75	9·75

* This experiment gives an unusually high value for the numbers of hyphae unbranched in distilled water. The cultures used were 44 hours old, which is younger than in the other experiments, and there is a general indication that fewer hyphae branch, on treatment with distilled water, in young than in old cultures.

All the solutions allowed almost all the hyphal apices to grow uninterruptedly, in contrast to the effect of distilled water. It appears therefore that solutions of approximately neutral, non-toxic salts and crystalloids at the same molar concentration as the mineral-sucrose solution will allow the hyphal tips to continue to extend. The case of sucrose is of particular interest because in previous experiments the hyphal apices had been affected by all the concentrations of sucrose investigated.

4. *In sucrose solutions $<$ 0·5 M concentration*

The results of an experiment in which solutions of sucrose $<$ 0·5 M concentration were applied to the hyphal apices of *F. oxysporum* are recorded graphically in Fig. 3.

It can be seen that the number of apices unaffected reaches a maximum with sucrose concentrations between 0·125 and 0·25 M, and high values are also recorded for 0·0625 M. This spread, allowing for the variations between treatments and between experiments (cf. Table 5) is sufficient to account for the fact that the majority of hyphal apices grow on unchecked in solutions of 0·076 M sucrose, and in the same concentration of mineral-sucrose solution and of mineral salts alone.

The number of apices showing branching gives an inverse reading except at the higher concentrations of sucrose where the number of apices showing regrowth by means of one subterminal branch becomes progressively greater and the number showing regrowth with two or more branches correspondingly less.

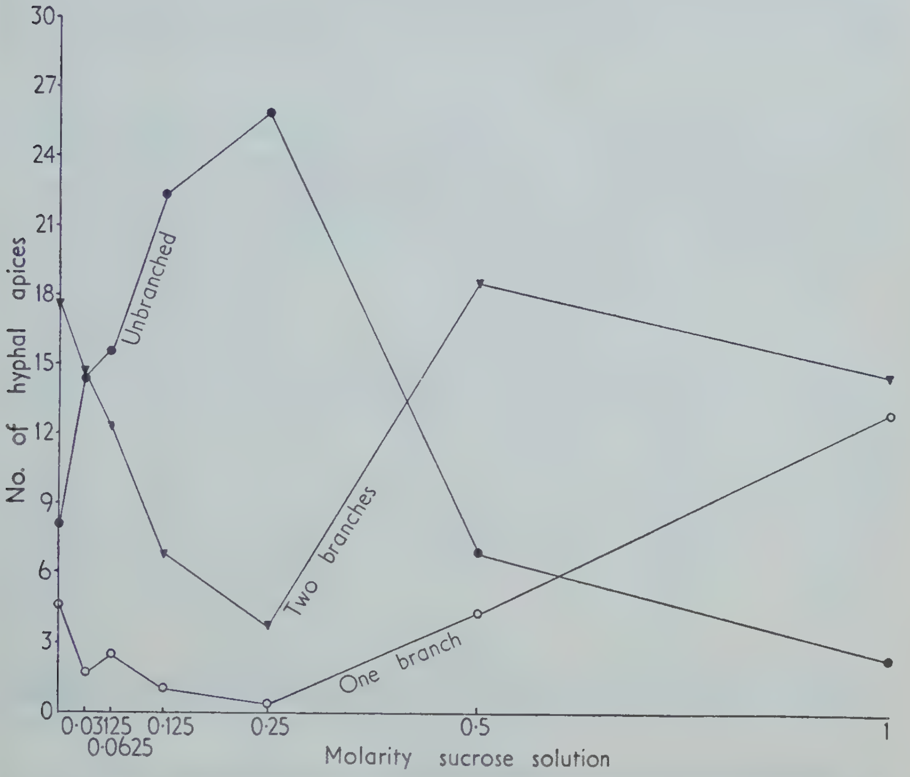


FIG. 3. Effect of molarity of bathing solutions of sucrose on the branching of the hyphal apices.

5. *On mineral-sucrose medium containing ten times the normal concentration of sucrose*

When the fungus was grown on a mineral-sucrose medium containing ten times the normal concentration of sucrose and was then exposed to a series of sucrose solutions of differing molarities the greatest number of hyphal apices was unaffected in the 0·75 M sucrose solution (Table 5). From this it can be concluded that the suction pressure of the hyphal apex varies with the medium on which it is grown.

TIME RELATIONS OF THE PHENOMENON

The interpretation of the results in terms of the osmotic pressure of the hyphal apex and of that of the bathing solution presents certain difficulties. If the stoppages of the extension of the hyphal apex in water and the subsequent branching is due to the entry of water causing swelling of the apical region and consequent derangement of the extension mechanism, why should a similar result be observed with solutions of sucrose of concentration greater than 0·25 M? Part of the difficulty of interpretation lies in the fact that sucrose or a product of its degradation, probably enters the hyphal apex to establish an equilibrium with the bathing fluid, and the rate at which this takes place is not known. However, the following experiments throw another light on the problem and make it unnecessary to consider the entry of sucrose in the early stages of the phenomenon. On the supposition that the apparent swelling of the hyphal apex, on immersion in water, was accompanied by stretching of the wall, an experiment was designed to discover at what point this stretching became permanent and how it might be related to the branching already discussed. Fungal colonies were immersed for short periods in water and then the water was replaced by mineral-sucrose solution (in which swelling does

TABLE 5

The Effect of Sucrose Solutions of Different Molarities on the Hyphal Apices of F. oxysporum *when grown on Mineral-sucrose Agar and Mineral-sucrose Agar with the Sucrose increased tenfold*

Mean of six observations, on samples of thirty hyphal apices from separate plates

Medium	Mineral-sucrose agar (0·076 M)			Mineral-sucrose agar with ×10 sucrose (0·472 M)		
	No. hyphal apices			No. hyphal apices		
Molarity sucrose solution	Unbranched	With one branch	With two branches	Unbranched	With one branch	With two branches
0 (Water)	16·3	3·3	10·3	Protoplasm retracted from wall		
0·03	17·3	2·3	10·3	,,	,,	,,
0·06	23·6	0·6	5·6	,,	,,	,,
0·125	27·3	0·0	2·6	,,	,,	,,
0·25	28·0	0·0	2·0	1·3	10·6	18·0
0·5	3·0	7·0	20·0	3·0	10·0	17·0
0·75	1·3	16·3	12·3	23·3	1·6	6·6
1·0	Reaction slow, not recorded			1·0	12·0	17·0
1·25	,,	,,	,,	0·0	20·3	9·6
1·5	,,	,,	,,	1·0	22·0	7·0

TABLE 6

Effect of Exposure of Hyphal Apices to Distilled Water and then to Mineral-sucrose Solution

Mean of two observations, on samples of thirty hyphal apices each, on one plate

Time of exposure to distilled water (min.)	Time of exposure to mineral-sucrose solution (min.)	No. hyphal apices unchanged	No. hyphal apices with one branch	No. hyphal apices with two branches
20	0	12·5	3·0	15·5
9	11	10·5	2·5	17·5
8	12	11·0	2·0	17·0
7	13	11·0	1·5	17·5
6	14	10·0	4·0	16·0
5	15	8·0	4·0	18·0
4	16	11·0	1·0	18·0
3	17	14·0	4·0	12·0
2	18	13·0	3·0	14·0
1	19	5·0	2·5	23·5
0	20	27·5	0·0	2·5

not normally take place). The mineral-sucrose solution was renewed twice in rapid succession to ensure that there was no dilution by residual water adhering to the colony. In the first experiment the fungal colonies were immersed in distilled water for periods of 0–10 minutes, before being transferred to the mineral-sucrose solution for 10 minutes or longer, except in the case of the distilled water control (Table 6).

It is clear from these figures that exposure to distilled water for as short a period as 1 minute is sufficient to stimulate the production of branches from hyphal apices. In the following experiment, therefore, an attempt was made to find how short an exposure to distilled water would stimulate the production of branches from the hyphal apex. The results are presented in Table 7 below.

TABLE 7

Effect of Exposure of Hyphal Apices to Distilled Water for Periods of less than 1 minute and then to mineral-sucrose solution

Mean of two observations, of thirty hyphal apices each, on one plate

Time of exposure to distilled water	Time of exposure to mineral-sucrose solution	No. hyphal apices unbranched	No. hyphal apices with one branch	No. hyphal apices with two or more branches
Momentary	20 min.	10·5	4·5	15·0
5 secs.	19 min. 55 secs.	1·0	3·0	26·0
10 secs.	19 min. 50 secs.	3·0	7·0	20·0
20 secs.	19 min. 40 secs.	0·5	5·0	24·5
40 secs.	19 min. 20 secs.	8·5	3·0	18·5
80 secs.	18 min. 40 secs.	11·5	5·5	13
20 min.	0 min.	12·5	2·5	15
0 min.	20 min.	28·5	0·0	1·5

TABLE 8

Effect of Immersion of Hyphal Apices in Distilled Water, for Varying Periods, followed by Immersion in 0·076 M KCl

Mean of five observations, of samples of thirty hyphal apices each, on separate plates

Time in distilled water	Number of apices		
	Unbranched	With one branch	With two branches
5 secs.	7·2	6·2	16·6
10 secs.	5·4	3·2	22·4
20 secs.	6·8	2·8	20·4
40 secs.	10·4	1·2	18·4
60 secs.	19·4	0·8	9·8
80 secs.	19·6	1·2	9·2
20 mins.	18·8	0·6	10·6

From this it can be seen that exposure of the hyphal apices to distilled water for as short a period as 5 seconds, when followed by immersion in mineral-sucrose solution, is sufficient to promote branching and furthermore appears to promote more branching than exposure to distilled water for 40 seconds or longer. This is confirmed in a further experiment, the results of which are given in Table 8. Here immersion in distilled water was followed by immersion in 0·076 M KCl.

Further experiments showed that the complete mineral-sucrose solution could be replaced by any one of its constituents at a concentration of 0·076 M. There was an indication that the sucrose solution was less effective than the others. This was confirmed in another experiment, of which the results are shown in Table 9. On the assumption that an important difference between the sucrose solution and the other constituents lay in the absence of ionization of the sucrose molecule and a consequent lower osmotic pressure of the solution, a treatment with sucrose at a concentration of 0·15 M was included. This treatment gave results closely comparable with the solutions of mineral salts at 0·076 M.

TABLE 9

Effect of Immersion of Hyphal Apices in Distilled Water and in Various Solutions

Mean of four observations, of samples of thirty hyphal apices each, on separate plates

	Number of hyphal apices		
Treatment	Unbranched	With one branch	With two branches
Distilled water	15·5	1	14·5
KCl, 0·076 M	29·25	0	0·75
Sucrose, 0·076 M	25·5	0	4·5
Sucrose, 0·152 M	28·25	0	1·75
Water 10 secs. followed by KCl, 0·076 M	1·25	2·5	26·25
Water 10 secs. followed by sucrose, 0·076 M	8·25	2·0	19·75
Water 10 secs. followed by sucrose, 0·152 M	2·25	2·25	25·5

THE RELATION BETWEEN SWELLING OF THE HYPHAL APEX AND BRANCHING

So far it would appear that immersion of the hyphae in water or solutions of sucrose or mineral salts, at appropriate concentrations, brings about (*a*) stoppage of forward extension; (*b*) swelling of the hyphal apex; and (*c*) production of forward-pointing branches from the apex. As yet we have no clear picture of how the swelling of the apex takes place and we have no evidence to show that swelling and branching are causally related. Two explanations for the swelling of the hyphal apex after the stoppage of extension immediately present themselves. The first and simplest is that water continues to enter the hyphal apex and the resulting turgor pressure brings about stretching of the wall. The second, which springs from our ignorance of the mechanism of hyphal extension, is that following stoppage of extension, wall substance continues to be formed and, being introduced into the wall at some distance behind the extreme apex of the hypha, leads to a localized growth of the wall with a consequent outward bulging. If this last or similar explanations not depending on turgor pressure were correct, the time taken for swelling of the hyphal apex would be independent of the osmotic pressure of the bathing solution. An experiment was therefore performed to compare the time to maximum swelling and the time to onset of branching in sucrose solutions of different molarities. The comparison was further extended to compare colonies immediately immersed in the sucrose solution with colonies first immersed in water for 10 seconds. The results are given in Table 10 below.

TABLE 10

Relationship between Time of Swelling and Time of Onset of Branching in Sucrose Solutions of Different Molarities

	Colonies immediately immersed in sucrose solution (one observation)		Colonies first immersed in water for 10 secs. (mean of three observations)	
Molarity of sucrose solution	Time to maximum swelling (minutes)	Time to onset of branching (minutes)	Time to maximum swelling (minutes)	Time to onset of branching (minutes)
0	1·5	6	1·53	6·6
0·03125	2·0	7	1·5	7·75
0·0625	4·0	7	3·3	6·6
0·125	No swelling	No branching	3·0	5·2
0·25	No swelling	No branching	5·7	6·9
0·5	(Not true swelling)	22	(Not true swelling)	27·1

From this experiment it can be seen that whereas the time to maximum swelling increases with increasing molarity of the bathing solution, the time to onset of branching remains more or less steady until the 0·5 M solution is reached. This suggests that in the lower molarities the time taken to swell the hyphal apex is dependent on the rate at which water is entering the apex and that turgor pressure and wall stretching can at least partly account for the swollen apex. These results also suggest that the mechanism controlling the production of branches from the apex is independent of the swelling. The coincidence of the time of swelling and time of branching in the 0·5 M sucrose solution is of interest. Very close observation of the hyphal apex shows that no true swelling takes place, the swelling noted in the experiment being due to the beginning of the outgrowth of the branch initials. The hyphal apices immediately immersed in the sucrose solutions behaved similarly to those first immersed in water except that no stoppage, swelling, or branching took place in the 0·125 and 0·25 M solutions.

It must also be noted that when colonies are transferred to the 0·125 and 0·25 M solutions, after immersion for 10 seconds in water, swelling takes place in the sucrose solutions even although without pre-treatment with water the sucrose solution would appear to be isotonic with the hyphal apices. This suggests that there is a difference between the behaviour of a temporarily arrested hypha and one which is increasing in length in the normal way.

The results of a series of experiments on the relationship between the time to maximum swelling and the time to onset of branching in sucrose solutions of differing molarities, after immersion for 5 seconds in water, are presented graphically in Fig. 4; they confirm the results of Table 10 but show that the curve is not a smooth one and probably represents the interaction of a number of responses of the hyphal apex to flooding with the various solutions.

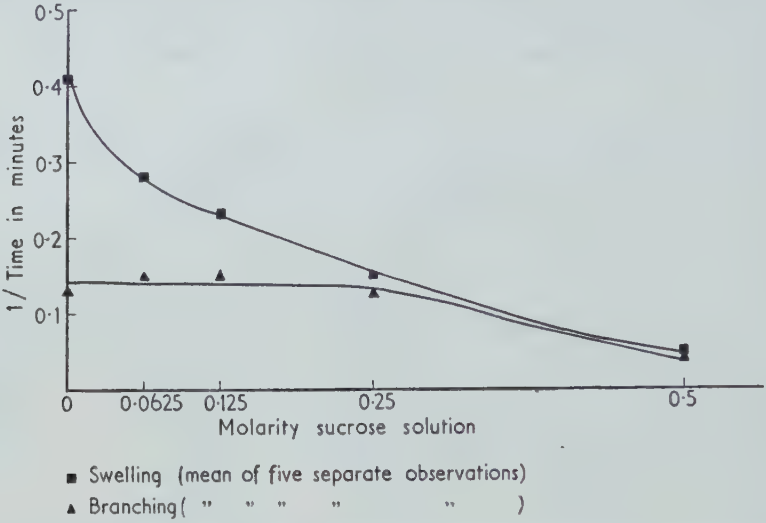


FIG. 4. Effect of molarity of the bathing solutions of sucrose on swelling and branching of the hyphal apices.

DISCUSSION

It is possible to conclude from the experimental data given here that in any colony of *F. oxysporum* there is a population of hyphal apices showing varying responses to solutions of differing molarities and that solutions of 0·125–0·25 M sucrose maintain the water relations of the greatest number of hyphal apices in the condition ordinarily met with at the surface of a plate of mineral-sucrose agar.

When a colony is flooded with distilled water all hyphae cease to elongate and all apices commence to swell, presumably because of the entry of water; a proportion rapidly recover and grow on, within 60 seconds, from the extreme tip. The other hyphal apices swell more and their forward growth is delayed for from 5 to 7 minutes; when it takes place it is by means of two or more forward pointing branches borne subterminally. The proportion of hyphae behaving in this way decreases in solutions of increasing molar concentrations of sucrose, from 0 to 0·125 M or in osmotically equivalent solutions of non-toxic mineral salts. This suggests that the suction pressure of the hyphae varies within the population. If such is the case the time at which the entering water is balanced by the increased pressure of the stretched wall should also differ in the population, if the extensibility of the wall is of the same order in all hyphae. On this view the hyphae with the smaller suction pressure will stop swelling before those with the higher suction pressure.

If this interpretation is correct it becomes possible to explain the results of the experiment where it is shown that exposure to distilled water for from 5 to 40 seconds, followed by exposure to mineral-sucrose solution (or its osmotic equivalent) brings about the branching of almost all the hyphal apices, whereas exposure to distilled water for 60 seconds or longer, before transfer, gives the same proportion of branched apices as exposure to distilled water alone. If the transfer takes place after 60 seconds the hyphal apices which have stopped swelling will already have commenced to grow on terminally and the resulting change in the rate of entry of water on transfer to mineral-sucrose solution will affect the new hyphal apex. No observable change can in fact be seen in the new apex on transfer, and it must be assumed that the change is not of a sufficient order to cause swelling or branching. If, however, the transfer takes place within 40 seconds and the new apical growing points are not yet reconstituted, the transfer to mineral-sucrose solution, involving a fresh equilibration, will effectively prolong the time for which the hyphal apices are arrested to the same order as that of the other hyphae with a higher suction pressure and a longer time of equilibration. From this it would appear that if hyphal apices are arrested for longer than 40 seconds a change takes place at the extreme tip which prevents renewed growth in this area. The new outlets for growth are then formed, after a delay of about 5 minutes, on the sides of the stretched apex.

The hyphae which do not grow on within 60 seconds, i.e. those hyphae which would branch in distilled water alone, might be expected to be affected by transfer to mineral-sucrose in a different way and to come more quickly to equilibrium and the point at which they can grow on. In fact the delay involved in the fresh equilibration, even although this is very rapid, still probably takes the apices past the point at which the irreversible changes in the wall at the extreme tip take place.

No simpler explanation of the phenomena reported has yet been found and more complicated explanations cannot be stretched to fit the other point which requires explanation, namely that hyphae immersed in solutions $> 0{\cdot}5$ M also react by branching but without swelling. On the supposition that arrestment of the hyphal apex for a period of more than 40 seconds is alone capable of promoting branching of the hyphal apex, the branching in hypertonic solutions can be explained by the delay in regrowth accompanying the loss of water and the equilibration of the hyphal tip in the sucrose solutions. Here, however, the time of arrestment is from 27 minutes to several hours depending on the strength of the solutions used.

Although this simple explanation is adequate to account for the branching of the hyphal apices under the various conditions mentioned it does not account for the swelling of the hyphal apices in 'isotonic' solutions (? and hypertonic solutions), after pre-treatment with water for from 10 to 40 seconds. The hyphal apices when growing normally are actively metabolizing and manufacturing new hyphal substance to provide for an extension rate of *c.* 5 μ per minute. When treated with water, although the extension mechanism is temporarily arrested, there is no evidence to show that the metabolic activities of the apex are also stopped. If metabolism is continuing without extension, the swelling of the hyphal apices in isotonic (? and hypertonic) solutions, after arrestment by pre-treatment with water, could result from the metabolic activity of the apex re-establishing a situation where water once more moves into the apex from the isotonic solution. This view is supported by the fact that the time at which swelling takes place is related to the molarity of the bathing solution in apices not pre-treated as well as in those pre-treated with water. It is possible to question why a point of equilibrium is not reached without swelling of the apex so that regrowth by means of branches follows immediately a certain rate of entry of water is achieved relative to the internal metabolic rate. The fact that in all treatments with solutions $< 0{\cdot}5$ M sucrose the time of onset of branching is more or less constant suggests that the organization of new hyphal apices, in the form of subterminal branches, requires a minimum length of time of the order of 7 minutes and until these branches have been organized there is no outlet for the metabolic products which therefore pile up and eventually by their osmotic effects promote entry of water and swelling of the apex.

No full explanation is available for the behaviour of the hyphal apices in solutions of sucrose $> 0{\cdot}5$ M where the number of apices with regrowth by means of one subterminal branch is greatly increased in the strongest solutions, but it is possible that after recovery in the high-sugar solutions the metabolism of the apex is reduced and only capable of providing for one new branch.

The behaviour of the hyphal apices recorded in this paper is by no means restricted to *F. oxysporum*. A survey of 27 spp. (sensu Wollenweber) of *Fusarium* showed that only five did not give any branched hyphal apices when the colonies on agar plates were flooded with distilled water; five showed less than 4/30 hyphal apices with two or more branches; eight gave 4–8/30 branched apices, and fourteen showed more than ten branched apices in the thirty apices observed. Only casual observations have been made on other fungi but practically all fungi examined show some degree of arrestment and swelling of the apex. So far, however, the marked dichotomous branching shown by the *Fusaria* has not been widely noted.

In the literature, branching of hyphal apices similar to that reported here for *F. oxysporum* is recorded for dysgonic strains of *Microsporum canis* by Johnstone and La Touche (1956) and in *Geotrichum* sp. by Nickerson *et al.* (1956).

The observations and techniques reported here have an obvious significance for further physiological studies on the fungi and they also show how a relatively simple system of arrestment followed by branching could be used to build up a wide variety of branch forms and conidiophore forms in the fungi.

ACKNOWLEDGEMENTS

I wish to thank many friends in the Cambridge Botany School who have discussed this problem with me and Miss Marion Scott for technical assistance.

LITERATURE CITED

BUXTON, E. W., 1955: The Taxonomy and Variation in Culture of *Fusarium oxysporum* from Gladiolus. Trans. Brit. mycol. Soc. **38,** 202.

CARLILE, M., 1956: A Study of the Factors influencing Non-genetic Variation in a strain of *Fusarium oxysporum.* J. Gen. Microbiol. **14,** 643.

JOHNSTONE, K. I., and LA TOUCHE, C. J., 1956: Cultural Characteristics of Dysgonic strains of *Microsporum canis* Bodin. Trans. Brit. mycol. Soc. **39,** 442.

NICKERSON, W. J., TABER, W. A., and FALCONE, G., 1956: Physiological Bases of Morphogenesis in Fungi. 5. Effect of Selenite and Tellurite on Cellular Division of Yeastlike Fungi. Can. J. microbiol. **2,** 575.

Interaction in growth and germination between Thiourea and Indolyl Acetic Acid

BY

A. POLJAKOFF-MAYBER, A. M. MAYER, AND S. ZACKS

(*Botany Department, Hebrew University and Research Council of Israel, Jerusalem*)

ABSTRACT

The action of indolyl acetic acid (IAA), of thiourea and their joint effect on the germination of lettuce seeds and the growth of the seedlings was investigated. Two different sources of the same variety of lettuce seeds were used. They differed greatly in their response to the substances tested. Thiourea stimulated germination and inhibited growth, except at one concentration, where stimulation of root growth was noted. IAA stimulated germination in certain cases. It did not affect either root or hypocotyl length. The effect of the treatments was modified by the temperature. No interaction of IAA and thiourea could be shown. Stimulation of germination is not a result of stimulation of growth.

THIOUREA has long been known as a compound active in various biological processes in animal and plant organisms. Thus its bacteriostatic effect has been described by De Ritis and Scalfi, 1946. Its effects on metabolism in animals have been reported (Du Bois *et al.*, 1947; Cornatzer *et al.*, 1953; Doljanski *et al.*, 1956). Thiourea is known to affect mitotis. It inhibits various oxidative enzyme systems (Du Bois and Erway, 1946; Kutacek, 1954; Sato and Niwa, 1952). Thiourea is also well known as an antioxidant used in industry.

In plants thiourea is chiefly known for its dormancy breaking and germination stimulating effect (Denny *et al.*, 1930; Thompson and Horn, 1944; Thompson and Kosar, 1939). In this laboratory the factors affecting the germination of light-dormant lettuce seeds and the biochemical changes occurring during the process have been investigated. In this work thiourea has been used as a germination stimulating substance. Its effect on various aspects of metabolism and germination has therefore been studied (Poljakoff-Mayber, 1952 *a*, *b*, 1953, 1955, 1957; Poljakoff-Mayber and Mayer, 1955; Mayer, 1954, 1956, 1957). In addition, preliminary experiments have shown that thiourea at germination stimulating concentrations inhibits root and hypocotyl growth of lettuce seedlings (Mayer, 1957).

Despite this none of the known effects of thiourea permit an interpretation of its effect on germination and growth. It was therefore decided to approach the problem from a different aspect, that of interaction of thiourea with other substances which affect germination and growth. A number of compounds were selected for investigation. In this paper it is intended to report on the joint action of thiourea and indolyl acetic acid (IAA) on growth of root and hypocotyl and on germination.

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

METHODS

The experiments were carried out on two lots of lettuce seed, variety 'Grand Rapids'. One lot referred to as 'imported' were seeds obtained from the Ferry Morse Seed Growers, California, in 1954. The second lot referred to as 'local' seeds were seeds harvested in 1954 from locally grown lettuce plants. The seeds were germinated in Petri-dishes on filter paper, using 3 ml. of water or test solution in the dark. IAA was used as its Na salt. Germination was determined after 72 hours' incubation. The length of root and hypocotyl of the intact germinated seedlings was determined at the same time. The same plants were used for the three determinations.

The statistical analysis was designed to detect main effects and the interaction of thiourea and IAA. A straightforward analysis of variance (Hald, 1952) could not be accomplished because of the non-orthogonality of the factors (Kendall, 1952). This was due to varying numbers of seeds in any replication and varying number of replications in different treatments. The hypothesis of independence was tested by means of regression analysis (Kendall, 1952). If this hypothesis was not rejected, then treatment means were separated into homogenous groups for every treatment with thiourea independently by the methods of Tukey (1949) and Duncan (1955).

The variates subjected to statistical analysis are the means of the values measured. In the case of percentage germination a $2 \arcsin \sqrt{p}$ transformation of the percentage of germination was carried out.

TABLE 1

The Action of IAA and Thiourea on the percentage Germination of Different Lots of Lettuce Seeds at Two Temperatures

Treatment	Thiourea concentration —M	IAA concentration —M				
		0	10^{-9}	10^{-8}	10^{-7}	10^{-6}
Local seeds 26° C. .	0	5·8	8·2	7·4	8·5	8·4
	$1{\cdot}0 \times 10^{-2}$	21·4	18·2	20·7	18·2	23·5
	$2{\cdot}5 \times 10^{-2}$	16·1	15·5	12·9	15·5	15·5
	$5{\cdot}0 \times 10^{-2}$	12·4	16·9	15·6	9·9	19·5
Local seeds 20° C. .	0	26·8	32·3	28·0	43·7	28·5
	$1{\cdot}0 \times 10^{-2}$	67·4	57·4	50·2	63·5	64·0
	$2{\cdot}5 \times 10^{-2}$	76·5	77·5	75·9	74·8	72·1
	$5{\cdot}0 \times 10^{-2}$	56·3	59·0	57·8	58·2	53·2
Imported seeds 26° C.	0	27·6	32·6	24·7	22·9	26·1
	$1{\cdot}0 \times 10^{-2}$	43·0	41·3	50·7	52·7	45·6
	$2{\cdot}5 \times 10^{-2}$	46·7	47·2	41·5	32·6	39·6
	$5{\cdot}0 \times 10^{-2}$	53·7	54·7	54·3	53·1	63·7

RESULTS

After preliminary experiments, the concentration ranges were chosen as follows: Thiourea 1×10^{-2}, $2{\cdot}5 \times 10^{-2}$ and 5×10^{-2}M, all of which stimulate germination and inhibit growth. The IAA concentration 10^{-9}, 10^{-8}, 10^{-7},

TABLE 2

Hypocotyl Length (in mm.) of Different Lots of Lettuce Seedlings at Two Temperatures and at Different Concentrations of IAA and Thiourea

Treatment	Thiourea concentration —M	IAA concentration —M				
		0	10^{-9}	10^{-8}	10^{-7}	10^{-6}
Local seeds 26° C.	0	4·3	7·0	5·9	6·6	6·9
	$1·0 \times 10^{-2}$	3·9	3·5	4·3	4·5	4·4
	$2·5 \times 10^{-2}$	3·3	3·1	3·1	3·3	2·8
	$5·0 \times 10^{-2}$	1·8	2·2	2·0	1·6	1·9
Local seeds 20° C.	0	3·3	3·1	2·7	3·3	2·8
	$1·0 \times 10^{-2}$	2·5	2·5	2·5	2·6	2·6
	$2·5 \times 10^{-2}$	2·8	2·6	2·8	2·4	2·6
	$5·0 \times 10^{-2}$	0·7	1·2	1·2	1·3	0·8
Imported seeds 26° C.	0	6·9	7·5	7·7	6·8	6·5
	$1·0 \times 10^{-2}$	3·7	4·4	4·9	4·4	4·1
	$2·5 \times 10^{-2}$	3·3	4·5	5·3	5·0	4·1
	$5·0 \times 10^{-2}$	1·7	1·6	1·6	1·5	1·7

TABLE 3

Root Length (in mm.) of Two Lots of Lettuce Seedlings at Two Temperatures, and Diverse Concentrations of IAA and Thiourea

Treatment	Thiourea concentration —M	IAA concentration —M				
		0	10^{-9}	10^{-8}	10^{-7}	10^{-6}
Local seeds 26° C.	0	4·6	7·2	6·2	6·9	7·6
	$1·0 \times 10^{-2}$	7·9	6·6	9·8	8·8	9·4
	$2·5 \times 10^{-2}$	3·6	4·3	3·2	3·1	3·1
	$5·0 \times 10^{-2}$	1·0	1·0	1·2	1·0	1·1
Local seeds 20° C.	0	8·9	9·3	8·4	8·6	8·8
	$1·0 \times 10^{-2}$	6·2	6·3	5·8	6·9	6·6
	$2·5 \times 10^{-2}$	2·6	3·3	3·5	2·7	2·7
	$5·0 \times 10^{-2}$	1·0	1·0	1·0	1·0	1·0
Imported seeds 26° C.	0	11·9	12·6	13·1	12·0	10·9
	$1·0 \times 10^{-2}$	7·0	8·4	7·0	7·4	7·0
	$2·5 \times 10^{-2}$	3·7	4·5	4·6	8·2	6·9
	$5·0 \times 10^{-2}$	1·3	1·5	1·6	1·3	1·3

and 10^{-6} M was chosen in the range which had been previously shown (Poljakoff-Mayber, 1957) to stimulate the germination of deep dormant lettuce seeds. Experiments with imported seeds were carried out at 26° C. only, at which the germination was around 30 per cent. Experiments with local seeds were carried out at 26° C., at which the germination was around 10 per cent., and at 20° C., at which germination was around 30 per cent., i.e. similar to that of the imported seeds at 26° C.

Table 1 summarizes the results in germination of the two lots of seeds at the different temperatures while Tables 2 and 3 give similar summaries for root and hypocotyl growth. Root and hypocotyl length was based on the average of germinated seeds only.

Table 4 summarizes homogeneous groups of treatment and characterizing parameters.

TABLE 4

Homogeneous Groups of Treatments and Characterizing Parameters

Observed variable	Seeds and temperature	Homogeneous groups of treatments	Means of homogeneous groups	95% confidence limits of estimated means	
				Upper	Lower
% of germination .	Local seeds 26° C.	Th. 1·0	19·9	23·2	16·9
		Th. 2·5; Th. 5·0	17·0	18·5	15·5
		Th. 0	7·0	8·0	6·0
	Local seeds 20° C.	Th. 2·5	77·4	80·0	74·8
		Th. 1·0; Th. 5·0	66·6	69·5	63·6
		Th. 0 (I.A.A. 10^{-7})	39·9	54·6	26·2
		Th. 0 (others)	27·1	30·6	23·7
	Imported seeds 26° C.	Th. 5·0	53·9	56·3	51·5
		Th. 1·0; Th. 2·5	43·2	46·7	39·7
		Th. 0	26·0	27·1	24·8
Root length (mm.)	Local seeds 26° C.	Th. 1·0	8·51	9·95	7·06
		Th. 0	6·45	7·26	5·64
		Th. 2·5	3·41	3·93	2·88
		Th. 5·0	1·09	1·12	1·06
	Local seeds 20° C.	Th. 0	9·17	9·69	8·66
		Th. 1·0	6·36	7·03	5·69
		Th. 2·5	2·94	3·26	2·61
		Th. 5·0	1·01	—	—
	Imported seeds 26° C.	Th. 0	12·19	12·81	11·57
		Th. 1·0	7·60	8·12	7·08
		Th. 2·5	5·58	6·72	4·45
		Th. 5·0	1·42	1·53	1·31
Stem length (mm.	Local seeds 26° C.	Th. 0	5·96	6·94	4·98
		Th. 1·0; Th. 2·5	4·02	4·42	3·62
		Th. 5·0	1·80	2·06	1·54
	Local seeds 20° C.	Th. 0	3·06	3·23	2·88
		Th. 1·0; Th. 2·5	2·55	2·69	2·40
		Th. 5·0	1·03	1·23	0·83
	Imported seeds 26° C.	Th. 0	7·02	7·35	6·68
		Th. 1·0; Th. 2·5	4·27	4·59	3·95
		Th. 5·0	1·60	1·65	1·54

(Th. 1·0, &c. = Thiourea 1, &c. × 10^{-2} M)

A number of facts emerge from a consideration of Table 4.

Germination. Local seeds were more sensitive to stimulation by thiourea than imported ones, the maximal stimulation being greater. In addition, the concentration required to bring about the stimulation was lower and varied with the temperature. Local seeds at 20° showed a response to an optimal thiourea concentration. IAA alone significantly increased germination of local seeds at 20° C. at 10^{-7} M. Its effect on germination of local seeds at 26° C. is, however, in doubt, being on the border of significance. The germination of imported seeds was not affected by IAA. There was no interaction between IAA and thiourea.

Hypocotyl growth. This was suppressed in all cases by rising thiourea concentrations. The hypocotyls of local seeds were shorter than those of imported seeds. They were longer at 26° C. than at 20° C. There was no interaction with IAA and IAA here did not affect hypocotyl length.

Root growth. The results differed from those in the previous groups, as here there were significant differences between all the thiourea concentrations. Germination and hypocotyl growth showed two of the thiourea concentrations to be not significantly different.

Whereas local seeds at 20° C. and imported seeds at 26° C. had root growth progressively depressed by increasing thiourea concentrations, this was not the case for local seeds at 26° C. The elongation of the latter was significantly stimulated by 1×10^{-2} M thiourea. It is also worth noting that the root length differed appreciably between the two lots of seeds and also between the two temperatures for the local seeds. Roots were longer at 20° C. than at 26° C.

Local seeds at 26° C. were also of interest, because of the interaction between thiourea and IAA. This interaction has been further analysed. An interaction model given by Finney (1952) was fitted, the model being:

$$Y_1 = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2$$

where x_1 and x_2 are metameters of the concentrations of IAA and thiourea respectively.

It was found, however, that the coefficients β_1 and β_2 were not significantly different from zero. It is therefore inferred that interaction occurred only at the stimulating thiourea concentration, i.e. 1×10^{-2} M. It was not affected by the IAA concentration.

DISCUSSION

From these results a number of points emerge. Firstly, it appears that the two lots of seeds, because of their difference in germination, also differed in the growth of their roots and hypocotyls in water. Moreover, the two lots of seeds also differed in their sensitivity to thiourea. The local seeds were more sensitive to thiourea, as far as germination is concerned, than the imported ones. For local seeds the maximal germination obtained with thiourea was from 2·5 to 3 times that observed in water. Imported seeds germinated only twice as much in thiourea as in water. In addition, the thiourea concentration required to bring about maximal germination was lower for local seeds than for the imported ones. For the local seeds a change of temperature also caused a change in sensitivity. The stimulation of germination by IAA previously reported for the same lot of local seeds (Poljakoff-Mayber, 1957) was again established. It was, however, much more evident at the lower temperature. The lowered sensitivity to IAA at the higher temperature may have been due to the additional storage period which the seeds had undergone. This apparently caused a slow emergence from the deep dormancy which is accompanied by wide fluctuations in the day-to-day germination percentage. Hence the IAA stimulation of germination was lower and its significance is doubtful.

Generally, stimulation of germination is accompanied by earlier and more rapid germination. Thus, it might be expected that higher germination in water would be followed by greater hypocotyl and root length. However, it appears that hypocotyl growth and germination have different temperature optima. Root growth and germination have similar optima. For root growth, therefore, increased germination appears to be accompanied by increased root length in local seeds, but not in imported ones. The latter had longer roots at 26° C. than the local ones at 20° C., despite the fact that the germination per cent. is of the same order. No similar effect exists for hypocotyl length.

Generally, hypocotyl growth is very sensitive to thiourea, being depressed by increasing concentrations. IAA, however, had no apparent effect.

The most outstanding point as far as root growth is concerned is the stimulation caused by 1×10^{-2} M thiourea for the local seeds at 26° C. Equally remarkable is the interaction of IAA and thiourea at 1×10^{-2} M for these seeds and the same temperature. This interaction exists despite the fact that IAA alone does not have any effect on root length. The nature of this interaction is not clear. At a lower temperature, 20° C., neither the interaction nor the stimulation of root growth exists.

In conclusion it can be stated that the stimulation of germination is not due to stimulation of growth and occurred despite growth inhibition. Germination and growth have again been shown to be entirely different processes which show different sensitivity to various external agents.

ACKNOWLEDGEMENT

Our thanks are due to Miss N. Krishmaro, for technical assistance during this work.

LITERATURE CITED

CORNATZER, W. E., GALLO, D. G., and DAVISON, J. P., 1953: Hormonal Effects on Phosphorylation in the Liver of the Rat. Proc. Soc. expt. biol. Med., **84**, 103.

DENNY, F. E., MILLER, L. P., and GUTHRIE, J. D., 1930: Enzyme Activities of Juice from Potatoes Treated with Chemicals that Break the Rest Period. Contr. Boyce Thompson Inst., **2**, 417.

DE RITIS, F., and SCALFI, L., 1946 *a*: Antibacterial Action of Thiourea. 3. Demonstration of its Bacteriostatic Nature. Bull. Soc. ital. biol. sper., **22**, 699.

—— ——1946 *b*: Antibacterial Action of Thiourea. 4. Influence of some Cultural Conditions. Ibid., 701.

—— —— 1946 *c*: Action of Thiourea on Bacterial Metabolism. Ibid., 703.

DOLJANSKI, F., ESHKOL, Z., GIVOL, D., KAUFMANN, E., and MARGOLIASH, E., 1956: The Effect of Large Doses of Thiourea on the Composition of the Liver and the Urine of Rats. J. Endocrinology, **13**, 141.

DU BOIS, K. P., and ERWAY, W. F., 1946: Studies on the Mechanism of Action of Thiourea and Related Compounds. 2. Inhibition of Oxidative Enzymes and Oxidation Catalysed by Copper. J. biol. Chem., **165**, 711.

—— HERRMANN, R. G., and ERWAY, W. F., 1947: Mechanism of Action of Thiourea and Related Compounds. 3. Effect of Acute Poisoning on Carbohydrate Metabolism. J. Pharmacol., **89**, 186.

DUNCAN, D. B., 1955: A Multiple Range and Multiple F-Test. Biometrics **11**, 1.

FINNEY, D. J., 1952: Probit Analysis. Cambridge University Press (2nd ed.).

HALD, A., 1952: Statistical Theory with Engineering Applications. John Willey & Sons, New York.

KENDALL, M. G., 1952: The Advanced Theory of Statistics. Charles Griffin & Co., London.

KUTACEK, N., 1954: Effect of Isothiourea Derivatives on Mono Amino Oxidases. Chem. List, **48**, 4902.

MAYER, A. M., 1954: Polyphenol Oxidases of Lettuce Seeds and their Inhibition. Enzymologia, **16**, 277.

—— 1956: The Action of Thiourea as a Germination Stimulator. J. Expt. Bot., **7**, 93.

—— 1957: The Formation of a Yellow Pigment in Lettuce Seedling Roots Treated with Thiourea. Ibid., **8**, 125.

POLJAKOFF-MAYBER, A., 1952 *a*: Changes in the Metabolism of Lettuce Seeds during Germination and its Inhibition. Palest. J. bot., Jerusalem, **5**, 180.

—— 1952 *b*: Germination Inhibitors and Plant Enzyme Systems. 1. Catalase. Bull. Res. Council Israel, **2**, 239.

—— 1953: Peroxidase Activity in Germinating Lettuce Seeds. Enzymologia, **16**, 122.

—— 1955: Oxidative Activity of Particles Prepared from Lettuce Seedlings. J. expt. Bot., **6**, 313.

—— 1957: The Effect of Auxin and Antiauxin on the Germination of Lettuce Seeds. Bull. Res. Council Israel. (In press.)

—— and MAYER, A. M., 1955: Some Quantitative Changes in the Fat Metabolism of Germinating Lettuce Seeds and their Inhibition by Coumarin. J. expt. Bot., **6**, 287.

SATO, R., and NIWA, M., 1952: Nitrate Reduction. 7. Reinvestigation on the Identity of the Enzyme with Cytochrome b. Bull. Chem. Soc. Japan, **25**, 202.

THOMPSON, R. C., and KOSAR, W. F., 1939: Germination Stimulation of Dormant Lettuce Seeds by Sulfur Compounds. Plant Phys., **14**, 567.

—— and HORN, N. L., 1944: Germination of Lettuce Seeds at High Temperature 25–35° C. Stimulated by Thiourea. Proc. Am. Soc. Hort. Sci., **45**, 431.

TUKEY, J. W., 1949: Comparing Individual Means. Biometrics, **5**, 99.

The Irradiation of Dividing Cells: Changes in Sensitivity to X-Rays during Mitosis

BY

DOUGLAS DAVIDSON

(*Department of Botany, University of Oxford*)

With three Figures in the Text

Abstract

Roots of *Vicia faba* were given combined X-ray and colchicine treatments. Breaks were not visible immediately after irradiation, even at metaphase. Breaks and reunions were, therefore, scored later in 2*x* nuclei, and in the 4*x* nuclei derived from 2*x* nuclei after colchicine treatment. It was shown that metaphase is the most sensitive stage of mitosis.

Breaks per chromosome are less frequent in 4*x* nuclei than in 2*x* nuclei after irradiation of resting stage. This apparent physiological difference between the two types of nuclei may affect either the frequency of breakage or of restitution. After the irradiation of prophase, metaphase, or telophase cells, however, breaks per chromosome are more frequent in the 4*x* nuclei than they are in 2*x* nuclei in the same sample owing to the fact that the 2*x*, unlike the 4*x* nuclei, are not accurately timed. They must include cells irradiated during the less sensitive resting stage.

Chromosome reunion occurred, probably at telophase. Chromatid reunion and sister reunion did not occur frequently. Chromatid breaks, like chromatid reunions, were infrequent except after irradiation of resting stage cells. The evidence indicates that the chromosome, at metaphase just as at resting stage, reacts to X-rays as a single functional unit.

Introduction

CHROMOSOME sensitivity to irradiation varies during the mitotic cycle. Maximum breakage, as observed at the following metaphase, is induced at the end of the resting stage. But with the beginning of spiralization, which is also the beginning of prophase, ordinary breakage ends. Special abnormalities occur when prophase is irradiated and they may involve the exchange of chromatid fractions. Finally, at meta-anaphase, no breaks are visible immediately after irradiation.

Following the irradiation of metaphase of mitosis (Conger, 1947) or meiosis (Sparrow, 1948) breaks are found in X_2 divisions. It appears that the free chromosomes are breakable but that breakage is deferred. The process of spiralization, it seems, does not prevent breakage but only its immediate analysis. It interferes with the separation of fragments by holding them together laterally.

The high frequency of breaks in X_2 divisions indicated that chromosome sensitivity to X-rays is at its maximum during metaphase. A similar peak of

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

sensitivity at meiotic metaphase was found when X-ray-induced dominant lethal mutations were measured (Russell and Russell, 1955).

In analysing X_2 metaphases, Conger found chromatid breaks and concluded that in mitosis the metaphase chromosome is effectively four-strand to X-rays. But neither the prophase chromosome in mitosis (Davidson, 1958) nor the metaphase chromosome in meiosis (Haque, 1952) have been found to be four-stranded. Furthermore, the differences in sensitivity between various stages of mitosis are much greater in *Allium* and *Trillium* (Sparrow, 1948) than in *Tradescantia* (Haque, 1952). The reactions of metaphase chromosomes and the comparative sensitivities of mitotic stages were therefore examined.

In carrying out experiments similar to those of Conger *Vicia faba* was preferred to *Allium cepa*. In *Allium*, heterochromatin is scanty (La Cour, 1952) and chromosome reunion occurs frequently. In *Vicia*, on the other hand, there are several heterochromatic segments. Their fusion during interphase prevents chromosome movement and apparently interferes with chromosome reunion; (R'') is rare. Chromatid reunion (R'), however, is quite frequent. Chromosome reunion does occur in *Vicia*, especially in X_2 divisions, if the breaks seen at the X_1 metaphase reunited at telophase before the heterochromatin had fused (McLeish, 1952). Thus in *Vicia* the type of reunion as well as the type of breakage found after irradiation of metaphase will serve as markers of the time when the breaks are revealed.

Labelling cells. In meiosis, the intervals between stages are long. Also, the divisions within an anther are synchronized; thus it is possible to treat cells at a known stage. In roots divisions are not synchronized. An irradiation acts on a mixture of all stages of mitosis, all overlapping. A study of the effects of X-rays on mitotic cells in division therefore depended upon finding a method of timing cells from X_1 to X_2 metaphases with or without labelling them.

The mean time of a mitotic cycle in *Vicia faba* roots has been variously estimated (Gray and Scholes, 1951; Howard and Pelc, 1951, 1952); the actual time for one mitosis could vary from about 16 to 27 hours (Gray and Scholes, 1951). Such a degree of variation limits the use of a time-scale. It does not give an accurate assessment of the phase of a cell at some previous time. Furthermore, as irradiation increases the variation already present sometimes by hastening and sometimes by delaying the onset of division (Darlington and La Cour, 1945; Howard and Pelc, 1952), an experiment to determine the sensitivity of mitotic stages to X-rays could not be based solely on a time-scale.

Methods of labelling cells have been devised. Cells have been labelled with micronuclei by combined chemical and X-ray treatments (Revell, 1952) or as binucleate cells (Kihlman, 1955). On the other hand, an 85 per cent. synchronization of division in protozoan cultures was achieved by using temperature changes (Zeuthen and Scherbaum, 1954). None of these methods was satisfactory for the present purpose, however, and the use of colchicine was considered.

The effects of colchicine. The results from experiments using colchicine alone confirmed previous results (Levan, 1938; Conger, 1947); they were:

(i) Mitosis is arrested at metaphase. This gives numbers of nuclei synchronized at one of the stages whose sensitivity was to be determined.

(ii) The arrested metaphase forms a single restitution nucleus which, at the following division, is tetraploid. It is thus labelled and easily distinguished from other cells.

(iii) The tetraploid nucleus has a known age under controlled conditions and therefore has its own developmental time-scale.

(iv) Arresting divisions at metaphase increases the synchronization of diploid nuclei and, one mitotic cycle later, of the tetraploid nuclei.

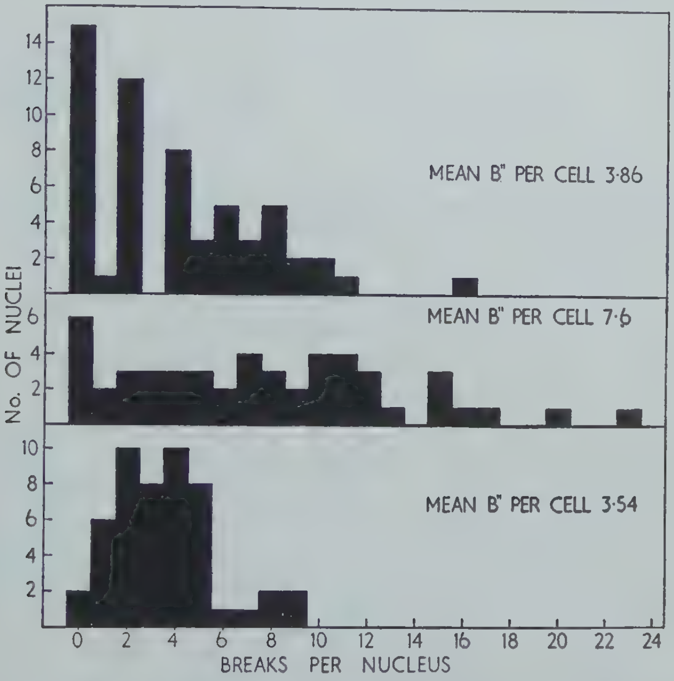


FIG. 1. Breakage and reunion in 2*x* and 4*x* cells of *Vicia* at various times after 150 r. The values for the 4*x* cells expressed in terms of the diploid complement. Data from Tables 2A and 2B.

The synchronization that colchicine induces is, of course, not exact; there is always a residue of variation in phase. Also, overlapping of stages is never completely eradicated. For example, after treatment, pre- and post-metaphase are mixed with full metaphases. This variation in stage probably contributes to the range of breaks shown by tetraploid nuclei (Fig. 1).

Colchicine alone has not been found to induce chromosome breaks, nor does it affect the frequency of breaks induced during the resting stage by X-rays (Davidson, 1957). It has been assumed that it does not affect the frequency of breaks induced by X-rays during metaphase.

MATERIAL AND METHODS

The culture conditions of the beans (*Vicia faba*: Carter's Aquadulce Longpod) have been described (Davidson, 1958). The cabinet temperature was 20° C. Treatments were started when laterals were 1 cm. long.

Roots were kept in 0·05 per cent. solution of colchicine for 3 hours. One and 2 hours' treatment did not result in sufficiently large numbers of tetraploid nuclei. Roots were thoroughly washed after treatment.

TABLE 1

Detailed Sequence of Combined X-ray (r) and Colchicine Treatments given to Roots of V. faba

Treatments	1	2	3	Sampling time
Prophase . .	150 r	Colchicine 3 hrs.	—	+24 and 27 hrs.
Metaphase . .	Colchicine 3 hrs.	150 r	—	+24 and 27 hrs.
Telophase . .	Colchicine 3 hrs.	0– 2 hrs.	150 r	+21 and 24 hrs.
Interphase . .	Colchicine 3 hrs.	0– 15 hrs.	150 r	+8 and 11 hrs.

A second colchicine treatment was given for the 3 hours before fixation. When it was intended to score tetraploid anaphases, the second colchicine treatment was omitted. Dividing cells, diploid and tetraploid, continued to be arrested at metaphase, however, owing to the effect of the colchicine. This effect continued for up to 30 hours, after the first treatment, and prevented the formation of anaphase figures in tetraploid cells.

The X-rays dose was 150 r in all experiments. It was given from a Newton Victor machine, unfiltered, in 75 seconds at 70 kp and 5 mA. The focal distance was 25 cm. Fixation: 2BD, diluted after 1 hour with an equal volume of 1 per cent. chromic acid. Bleaching and softening: mixture of equal volumes of 20 volumes H_2O_2 and saturated aqueous ammonium oxalate, for 10 minutes. Hydrolysis: in N . HCl for 12 minutes at 60° C. Staining: Feulgen. Preparation: as squashes in aceto-carmine. This stains the cytoplasm and helps to differentiate between *4x* and two adjacent *2x* cells.

The symbols used in scoring are those of Darlington and La Cour (1945). They are, however, used in terms of the X_2 division. Breaks induced at X_1 divisions and appearing at telophase will be scored as B'' and reunions as R''.

In all experiments two successive fixations, 3 hours apart, were made. This ensured that if those cells which suffered the least damage were the first to enter division, the later and perhaps more heavily damaged sample would not be missed. The results from the two fixations were, however, not markedly different.

PROPHASE–TELOPHASE IRRADIATION

(*a*) Diploid Cells

In all experiments the diploid nuclei in root-tips with tetraploid nuclei were also scored for *B* and *R*. The tabular data have been separated (Tables 2A and 2B). Corresponding horizontal lines in the two sections of the table give the data for the two types of cells from the same root.

TABLE 2A

Breakage (B) and Reunion (R) in Tetraploid Nuclei of Vicia *Roots after 150 r and Colchicine Treatment*

	Hrs. after X-raying	Metaphases		Total $B+R$				$B+R$ per cell (total scorable)				
		Total scorable	Broken	B'	B''	R'	R''	B'	B''	R'	R''	ECR
Prophase .	27	26	22	—	114	—	28	—	4·38	—	1·07	0·23
	30	33	22	—	114	—	25	—	3·45	—	0·76	
Metaphase .	24	26 (7)*	22	4	199	3	33	0·15	7·65	0·11	1·27	0·15
	27	24 (5)	22	—	181	—	25	—	7·54	—	1·04	
Telophase .	21	26 (2)	24	5	88	6	12	0·19	3·38	0·23	0·46	0·14
	24	24 (2)	22	7	89	4	13	0·29	3·71	0·16	0·54	
Interphase .	8	24 (3)	22	99	42	57	—	4·12	1·75	2·41	—	—
	10	23 (2)	22	91	39	68	—	3·95	1·69	2·95	—	—

* Unscorable.

TABLE 2B

Breakage (B) and Reunion (R) in Diploid Nuclei of Vicia *Roots after 150 r and Colchicine Treatment*

	Hrs. after X-raying	Metaphases		Total $B+R$				$B+R$ per cell (total scorable)			
		Total scorable	Broken	B'	B''	R'	R''	B'	B''	R'	R''
Prophase .	27	47	23	1	36	—	3	0·02	0·76	—	0·06
	30	48	23	12	36	14	5	0·25	0·75	0·29	0·1
Metaphase .	24	27	22	10	35	10	1	0·37	1·29	0·37	0·04
	27	50	30	10	52	6	2	0·02	1·04	0·12	0·04
Telophase .	21	34	24	13	32	13	—	0·38	0·94	0·38	—
	24	54	28	10	53	10	—	0·18	0·98	0·18	—
Interphase .	8	25	22	76	25	47	—	3·04	1·0	1·88	—
	10	25 (2)*	22	77	25	38	—	3·08	1·0	1·52	—

* Unscorable.

The mean breakage per nucleus was small. It is less than that in tetraploid cells irradiated at the same time in the same root, even when the values have been made strictly comparable by correction (Fig. 2). It is also less than the mean breakage per cell after pre-prophase irradiation (Fig. 2). Dicentric chromosomes were found. They occurred in low frequency, as in other experiments on *Vicia*, indicating that chromosome reunion (R'') was rare.

(*b*) Tetraploid Cells

These cells are derived from the restitution nuclei formed by colchicine-arrested metaphases in diploid cells.

Breakage. Breaks were almost wholly chromosomal (B''); there were few chromatid breaks (B'). The low frequency of B' shows that most breaks appeared before chromosome splitting took place and that after it there was little chromatid restitution. Such restitution would, of course, convert B'' into B'. The same result would follow from a chromatid reunion (R') between two chromosome fragments. R', therefore, also occurred only in low frequency. In one fixation, however, it exceeded B'. This was the first fixation after irradiation at telophase. The R' value per cell was 0·23, the B' value 0·19. Here, with low frequencies of B and R, the presence of a single tri-radial ($B''+B'+2R'$), has reversed the usual relation of B' and R' frequencies.

The highest mean value of B'' per cell and the greatest range of variation of B'' per cell occur after metaphase irradiation (Table 2A and Fig. 1). The mean value of B'' per cell is twice the values found after prophase or telophase irradiations. Metaphase is the stage in the mitotic cycle when the chromosomes show the maximum sensitivity to irradiation.

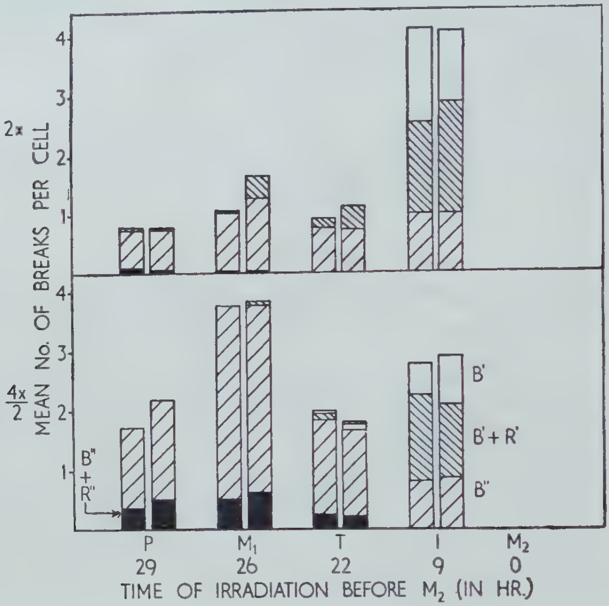


FIG. 2. Frequency distribution of breaks in 4*x* cells of *Vicia* after irradiation at telophase or at the previous diploid prophase or metaphase. 150 r.

The lowest range of variation of B'' per cell occurs after irradiating telophase (Fig. 1). B'' then follows a Poisson distribution ($\chi^2 = 1{\cdot}8$, $df = 4$, P is 0·7–0·8). Prophase and metaphase irradiations do not result in a Poisson distribution of chromosome breaks ($\chi^2 = 45{\cdot}9$, $df = 5$, $P < 0{\cdot}001$ and $\chi^2 = 34{\cdot}4$, $df = 5$, $P < 0{\cdot}001$ for prophase and metaphase respectively). These data show that the response to irradiation is fairly uniform at telophase. They also probably reflect the fact that telophase is relatively easily separated from other stages of mitosis, while prophase and metaphase are separated only with difficulty.

Reunion. The most frequent type of reunion was chromosome reunion (R''). Chromatid reunion (R') appeared sporadically, always in low frequency. Sister-reunion (*SR*) occurred once after a prophase irradiation. It was in an acentric fragment. The few chromatid reunions and the single sister-reunion that did occur show that R' is not prevented by healing of broken ends. The nucleus apparently exerted a different kind of control against reunion. This control operates after chromosome splitting. Since the frequencies of R' and *SR* are low, it is evident that breaks develop before the time of splitting and that after it R' and *SR* are almost prevented. It also indicates that R'', which occurs often, probably took place when the chromosomes were unsplit.

There are three possible types of reunion between centric (C_1) and acentric (C_0) fragments:

(i) C_1 with C_1, this forms a dicentric chromosome (C_2'');
(ii) C_1 with C_0, monocentric;
(iii) C_0 with C_0, acentric.

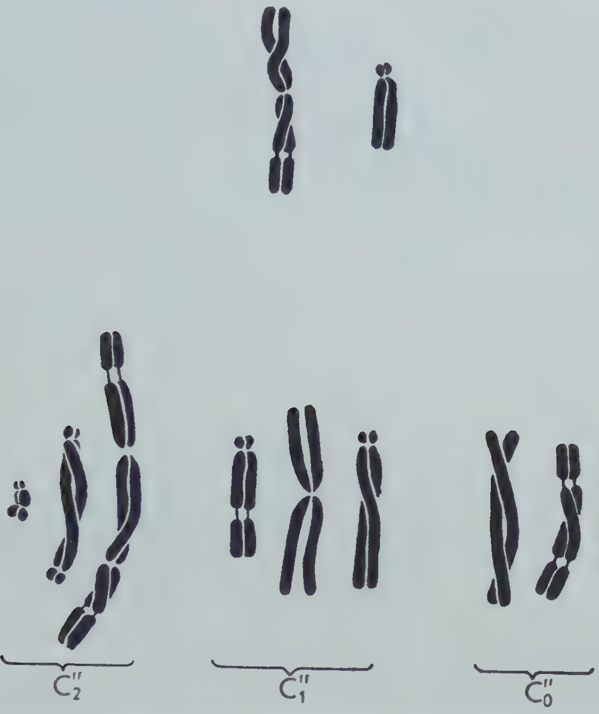


FIG. 3. Above, normal *M* and *S* chromosomes of *Vicia faba*. Below, new chromosomes or fragments formed after chromosome breakage and reunion. C_2'', dicentric; C_1'', centric; and C_0'', acentric. × 1,600.

Dicentric chromosomes are easily recognized. Reunions in mono- and a-centric fragments, however, can usually be recognized only when the reunion has resulted in an arm or in a fragment which is longer than the normal arm of the same type of chromosome. Other reunions would not be distinguishable. Thus, a value for R'' must always be an under-estimate. All R'' (Table 2) has been scored only from dicentric chromosomes; data from reunions giving rise to C_1 or C_0 fragments of exceptional length have not been included, though such reunions did occur (Fig. 3).

The empirical coefficient of reunion (*ECR″* or R''/B'', Darlington and La Cour, 1945) is used to relate R'' to B''. The highest value (Table 2A) in the present experiments occurs in cells treated at prophase, cells which did not have the highest frequency of breaks. Comparing cells treated at prophase and telophase reveals that although their breakage frequencies are almost equal, the number of dicentric chromosomes, and thus the *ECR″*, is halved in the later stage of mitosis. This fall in R'' per cell indicates that the time available for reunion is significantly reduced when the breaks are actually induced during telophase. It also shows that reunion occurs at the beginning of telophase, before the fusion of heterochromatic segments.

INTERPHASE IRRADIATION: DIPLOID AND TETRAPLOID CELLS

For the first time in these experiments breakage frequencies in diploid cells approach those in tetraploid cells (Tables 2A and 2B). Chromatid breaks are frequent. In diploid and tetraploid cells, they are more frequent than chromosome breaks. In diploid cells of bean roots B' is more frequent than B'' when the irradiation is given after chromosome reproduction (Thoday, 1954). The excess of B' over B'' in tetraploid cells indicates that chromosome reproduction or splitting had taken place some time in the 15-hour period between the end of the colchicine treatment and the time of irradiation.

Dicentric chromosomes were not found in the cells, diploid or tetraploid, irradiated during interphase. Their absence supports the view that reunion (R'') occurs during early telophase.

BREAKAGE AND REUNION

The frequency of breaks found in tetraploid metaphases confirms that in mitosis, as in meiosis, maximum sensitivity to X-rays is shown by metaphase chromosomes. But at metaphase or anaphase in the treated diploid cells, up to 15 minutes after X-raying, breaks are not visible. This is true if the dose is 100 or 250 r. The breaks, the results of X-raying, appear one mitotic cycle later.

The actual effects induced at the time of irradiation may be:

(i) Metastable loci which may later revert to a stable state with or without the formation of visible breaks;
(ii) Breakage in sub-chromatid fractions;
(iii) Immediate breakage, hidden by the contracted state of the chromosomes and by the lateral attraction of the spiralized parts.

Breaks should develop from labile loci (Auerbach, 1949; Östergren and Wakonig, 1954) independently of the time of chromosome splitting. The frequency of B'', and the almost complete absence of B' from cells irradiated during mitosis, indicate, however, that whatever changes are induced, breaks develop before chromosome splitting. There is no evidence that metastable loci are induced; that sub-chromatid breakage occurs; or that the metaphase chromosome is four-strand (cf. Conger, 1947). The evidence indicates that breakage is immediate but remains hidden while the chromosomes are contracted.

B'' is the predominant type of break in cells irradiated during mitosis. This establishes that the appearance of the breaks precedes chromosome splitting. The actual time of chromosome splitting in tetraploid cells can be estimated from the results of irradiating during interphase. B' is more frequent than

B'', showing that chromosome splitting or reproduction had occurred, or occurs about 15 hours after metaphase. In diploid cells, *DNA* synthesis occurs about 14 hours after metaphase (Howard and Pelc, 1951).

R'', measured as C_2'', occurs only in cells with high B'' frequencies. It is absent from cells with high B' frequencies. R'' therefore, like B'', occurs before chromosome splitting. The fall in the values of ECR'' from prophase to telophase irradiation also indicates that R'' occurs before chromosome splitting, probably during early telophase and immediately after the breaks are released by chromosome uncoiling.

The likely sequence of events is that breaks appear and undergo reunion during early telophase, i.e. as B' and R'. R' becomes less frequent as heterochromatic fusions occur. Chromosome reproduction and splitting follow, converting B' and R' into B'' and R''. After chromosome splitting almost no reunion occurs; R' and SR therefore are infrequent.

A similar situation occurred in X_2 metaphases in *Hyacinthus* (La Cour, 1952). B'' and R'' were frequent; B', R', and SR' were almost absent. Ends, newly broken owing to anaphase breakage, rejoined at early telophase before the formation of chromocentres. The absence of chromocentres during early telophase is a special condition which facilitates reunion in *Hyacinthus* and *Vicia*. After treatments which do not produce breaks during telophase, dicentric chromosomes do not occur in X_1 metaphases in *Vicia*, or they are infrequent (Table 2A; Darlington and La Cour, 1945; Revell, 1952; McLeish, 1952; Wolff and Luippold, 1955).

COMPARATIVE SENSITIVITIES OF DIPLOID AND TETRAPLOID CELLS

Diploid and the induced tetraploid metaphases occur in the same root. Halving B' and B'' values in the tetraploid nuclei measures breakage in terms of the diploid complement and makes an exact comparison between the two types of nuclei possible. The corrected values from tetraploid cells are greater than those from diploid cells, except after interphase irradiation. At this stage the tetraploid nuclei are less sensitive than the diploid nuclei. At all other stages they are more sensitive (Fig. 2).

The differences in the breakage frequencies in diploid and tetraploid cells emphasizes again the high sensitivity of dividing cells. But they give only an indication of the true relative sensitivities of diploid and tetraploid cells. For a valid comparison it is necessary to have both types of cells together and to irradiate them at the same stage (cf. Bishop, 1953). The interphase irradiation of diploid and tetraploid cells approximates to these conditions. At other times, the ploidy of the cell has been changed subsequent to irradiation. This may alter breakage frequencies; for example, by altering the frequency of restitution (p. 192).

FACTORS AFFECTING SENSITIVITY IN DIVIDING CELLS

The density of the chromosomes is greatest at metaphase as this is the stage of maximum contraction. The direct effect of X-rays may then be more important than it is during interphase (see Butler, 1957). The increase in density is probably due to contraction and not to synthesis. *DNA* synthesis, for example, ceases before the onset of prophase yet sensitivity continues to increase after it has finished (cf. Sparrow, Moses, and Steele, 1952; Sparrow, Moses, and Dubow, 1953).

The intra-cellular concentration of oxygen during irradiation delivered during resting stage is known to be important (Thoday and Read, 1949); sensitivity is minimum in the absence of oxygen; adding oxygen after X-raying does not increase the breakage frequency (Giles and Riley, 1950; Hollaender *et al.*, 1951). Now aerobic respiration in plant tissues falls during prophase (Erickson, 1947; Stern and Kirk, 1948), reaching a minimum at metaphase. Thus, the intracellular concentration of oxygen available to interact with X-rays, is probably highest at metaphase; this may be an important factor in determining the sensitivity of dividing cells.

During interphase, nuclei lack terminal oxidases (Allfrey, Mirsky, and Stern, 1955) and they may not contain dissolved oxygen. Indeed, the nuclear membrane may serve to reduce interaction with dissolved oxygen to a minimum (Stern and Timonen, 1954). The site of interaction between oxygen and X-rays must then be the cytoplasm. The important role played by cytoplasm is of course known (Duryee, 1949). It must also mean that mutagenic substances which form in the cytoplasm are later transferred to the nucleus (Giles, 1954; Hollaender *et al.*, 1951). At metaphase, however, circumstances are different. The nuclear membrane has broken down and there is no barrier between the cytoplasm and chromosomes. A rapid reaction between the products of irradiation and the metaphase chromosomes is possible. This will enhance the possible effect of other factors such as oxygen concentration.

RESTITUTION: METABOLIC ACTIVITY

The number of breaks induced by X-rays may be greatly reduced by subsequent restitution. Restitution occurs during interphase, when the cell is metabolically active. Restitutive processes may work with a diminished efficiency at telophase when the breaks induced during resting stage appear. A low frequency of telophase restitution could be partly responsible for the high sensitivity to X-rays in dividing cells but it would not account for higher sensitivity at metaphase than at prophase. Further, there is evidence that far from being low, telophase restitution in roots may be high.

In anthers diploid pollen grains have fewer breaks than haploid pollen grains though both are derived from the same pollen mother cells X-rayed at *MI* (Haque, 1952). A decrease in cell ploidy accompanies an increase in the number of breaks per cell. The modification of the number of breaks can have occurred only in the interphase following irradiation; it must have been achieved by restitution. In roots the breaks induced in diploid cells are seen in the tetraploid cells which form after colchicine treatment. As in anthers, there is a change in ploidy subsequent to irradiation. The higher degree of ploidy in root cells may result in more restitution and thus in lower breakage frequencies. This would reduce the difference between breakage frequencies in diploid and tetraploid cells.

In future it may prove possible to avoid changes in cell ploidy. The estimates of the relative sensitivities of different stages of division will then be better. Present estimates vary. Metaphase may be 30 times more sensitive than the least sensitive stage (Conger, 1947; Sparrow *et al.*, 1952); or, in meiosis, 3·5 times more sensitive than pachytene (Haque, 1952); I find mitotic metaphase is 2·1 times more sensitive than telophase and 4·5 times more sensitive than interphase. These variations in sensitivity between different stages of division indicate than the processes of breakage, restitution, and reunion, are probably affected by changes in cell metabolism.

SUMMARY

1. A single treatment with colchicine has three effects on root-tip mitosis in *Vicia*, as in *Allium*:
   - (*a*) It prolongs metaphase in diploid ($2x$) nuclei.
   - (*b*) It leads to the formation of tetraploid ($4x$) nuclei.
   - (*c*) These return to a second prolonged metaphase.
2. By X-raying root-tips before and after a short colchicine treatment it was possible to study the effects of irradiation at restricted times before, during, and after metaphase. It was possible to know that $4x$ nuclei, scored for breakage and reunion, had been derived from $2x$ nuclei treated at these restricted times, while sister $2x$ nuclei had been treated at mixed cell stages.
3. In the $4x$ nuclei the breakage frequency per chromosome was higher than in $2x$ nuclei of the same root. But when X-rayed 15 hours after colchicine treatment, that is, when $4x$ and $2x$ nuclei were treated in interphase, the $4x$ nuclei had a lower breakage frequency per chromosome than the $2x$ nuclei.
4. Breakage takes place in the spiralized chromosomes just before, during, and after metaphase but the breaks do not appear while the chromosomes are contracted; they are released by uncoiling. There is no evidence of variably delayed breakage with variable reunion which would follow the induction of metastable loci.
5. Breakage is released at telophase and is followed by reunion. Heterochromatin, which prevents R'' following interphase irradiation in *Vicia*, also reduces telophase reunion as compared with the frequency in plants without heterochromatin.
6. Metaphase, the most sensitive stage, is about twice as sensitive as telophase. As metaphase shows the greatest range of breaks per cell, it is probable that the real sensitivity is higher than the averages indicate.
7. The X_2 metaphases following prophase treatments should include chromosomes that would have shown sub-chromatid effects at X_1 anaphase. No vestiges of this type of breakage, however, were seen. Also, there was no evidence of dicentric chromosomes derived from SR of chromatid ends.
8. Breakage and reunion in prophase to telophase experiments are mainly B'' and R''; B', R', and SR occur in low frequency. In the interphase experiment

there is no R''; B' and B'' occur with R'. This confirms the view that B' can arise from B'' following restitution of one chromatid after splitting.

ACKNOWLEDGEMENTS

The present study was carried out with X-ray equipment purchased with a Scientific Investigations Grant-In-Aid of the Royal Society.

I am grateful to Professor C. D. Darlington for his helpful criticism; also to the Agricultural Research Council for the award of a scholarship.

LITERATURE CITED

ALLFREY, V. G., MIRSKY, A. E., and STERN, H., 1955: Chemistry of the Cell Nucleus. Adv. in Enzymology, **16.**

AUERBACH, C., 1949: Chemical Mutagenesis. Biol. Rev., **24,** 355–91.

BISHOP, C. J., 1953: The Influence of Polyploidy on the X-ray sensitivity of cells. Can. J. Bot., **30,** 139–46.

BUTLER, J. A. V., 1957: Effects of X-rays and Radiomimetic Agents on Nucleic Acids and Nucleoproteins. Ionizing Radiations and Cell Metabolism. Ciba Found. Symp. ed. G. E. W. Wolstenholme and C. M. O'Connor, 59–69.

CONGER, A. D., 1947: X-ray Sensitivity of the Stages of Mitosis. Amer. J. Bot., **34,** 582.

DARLINGTON, C. D., and LA COUR, L. F., 1945: Chromosome Breakage and the Nucleic Acid Cycle. J. Gen., **46,** 180–267.

DAVIDSON, D., 1957: Relation of Colchicine to Chromosome Breakage. Nature, **180,** 1059–60.

—— 1958: Chromosoma (in press).

DURYEE, W. R., 1949: Nature of Radiation Injury to Amphibian Cell Nuclei. J. Nat. Can. Inst., **10,** 735–95.

ERICKSON, R. O., 1947: Respiration of Developing Anthers. Nature, **159,** 275–6.

GILES, N. H., 1954: Radiation-induced Chromosome Aberrations in *Tradescantia.* Rad. Biol., **1,** 713–61.

—— and RILEY, H. P., 1950: Studies on the Mechanism of the Oxygen Effect on the Sensitivity of *Tradescantia* Chromosomes. Proc. Nat. Acad. Sci., **36,** 337–44.

GRAY, L. H., and SCHOLES, M. E., 1951: The Effect of Ionizing Radiation on the Broad Bean Root. Brit. J. Radiol., **24,** 82–92.

HAQUE, A., 1952: The Irradiation of Meiosis in *Tradescantia.* Heredity (Suppl.), **6,** 57–75.

HOLLAENDER, A., BAKER, W. K., and ANDERSON, E. H., 1951: Effect of Oxygen Tension and Certain Chemicals on the X-ray Sensitivity of Mutation Production and Survival. Cold Spring Harbor Symp., **16,** 315–26.

HOWARD, A., and PELC, S. R., 1951: Synthesis of DNA and Nuclear Incorporation of ^{35}S as Shown by Autoradiography. Isotopes in Biochemistry (CIBA), 138–47.

—— —— 1952: Synthesis of DNA in Normal and Irradiated Cells. Heredity (Suppl.), **6,** 261–73.

KIHLMAN, B., 1955: Chromosome Breakage in *Allium* by 8-EOC and X-rays. Exp. Cell Res., **8,** 345–68.

LA COUR, L. F., 1952: The Physiology of Chromosome Breakage and Reunion in *Hyacinthus.* Heredity (Suppl.), **6,** 163–79.

LEVAN, A., 1938: The Effect of Colchicine on Root Mitosis in *Allium.* Hereditas, **24,** 470–86.

MCLEISH, J., 1952: The Action of Maleic Hydrazide on *Vicia.* Heredity (Suppl.), **6,** 125–47.

ÖSTERGREN, G., and WAKONIG, T., 1954: True or Apparent Sub-chromatid Breakage and the Induction of Labile States in Cytological Chromosome Loci. Bot. Not., 357–75.

REVELL, S. H., 1952: Chromosome Breakage by X-rays and Radiomimetic Substances in *Vicia.* Heredity (Suppl.), **6,** 107–25.

RUSSELL, L. B., and RUSSELL, W. L., 1955: *see* EBERT, M., and HOWARD, A., 1955: Progress in Radiobiology. Nature, **176,** 683.

SPARROW, A. H., 1948: Relative X-ray Sensitivity of Metaphase and Interphase Chromosomes. Nature, **162,** 651.

—— MOSES, M. J., and DUBOW, R. J., 1953: Relationships between Ionizing Radiation, Chromosome Breakage and Certain other Nuclear Disturbances. Exp. Cell Res. Suppl., **2,** 245–67.

SPARROW, A. H., MOSES, M. J., and STEELE, R., 1952: A Cytological and Cytochemical Approach to an Understanding of Radiation Damage in Living Cells. Brit. J. Radiol., **25**, 183–9.

STERN, H., and KIRK, P. L., 1948: The Oxygen Consumption of the Microspores of *Trillium* in Relation to the Mitotic Cycle. J. Gen. Physiol. **31**, 243–52.

—— and TIMONEN, S., 1954: The Position of the Cell Nucleus in Pathways of Hydrogen Transfer. J. Gen. Physiol., **38**, 41–52.

THODAY, J., 1954: Radiation-induced Chromosome Breakage, DNA Synthesis and the Mitotic Cycle in Root Meristem Cells of *Vicia faba*. New Phytol., **53**, 511–16.

—— and READ, J., 1949: Effect of Oxygen on the Frequency of Chromosome Aberrations Produced by Alpha-rays. Nature, **163**, 133–4.

WOLFF, S., and LUIPPOLD, H. E., 1955: Metabolism and Chromosome-Break Rejoining. Science, **122**, 231–2.

ZEUTHEN, E., and SCHERBAUM, O., 1954: Synchronous Division in Mass Cultures of the Ciliate Protozoan *Tetrahymena pyriformis*, as induced by Temperature Changes. Colston Symp., **7**, 141–56.

Isolated Megaspore Tetrads of *Stauropteris burntislandica*

BY

W. G. CHALONER

(*Botany Department, University College, London*)

With Plate and one Figure in the text

Abstract

A new type of fossil spore tetrad, *Didymosporites scotti* gen. et sp. nov. is described from the Dinantian and Namurian (Carboniferous) of Ireland, Scotland, and England. The spores always occur in the form of a tetrad composed of two large (presumably fertile) and two minute (presumably abortive) spores, all with cutinized walls. Reasons are given for regarding these as the megaspores of the coenopterid fern *Stauropteris burntislandica*; their distribution indicates a far wider range in time and space than that previously known for this species. Their occurrence as adhering tetrads suggests that *Stauropteris burntislandica* 'shed' its megaspores still enclosed in the sporangium—an interesting analogy with both *Lepidocarpon* and the seeds of Pteridosperms.

Introduction

STAUROPTERIS is a genus of Carboniferous coenopterid fern based on petrifactions of the rachis and its branches. There are two well-established species: *S. oldhamia* from the Upper Carboniferous of England and *S. burntislandica* from the Lower Carboniferous of Scotland. (Darrah, 1941, has reported an American species but this has not been fully described.) D. H. Scott (1906) described microsporangia of the former species containing microspores germinating to produce rhizoids, and *Stauropteris* was accordingly accepted as a homosporous fern. Mrs. R. Scott (1908) described as *Bensonites fusiformis* some bodies which she regarded as glands found in connexion with *S. burntislandica*; Surange (1952) has subsequently shown these to be megasporangia containing two megaspores.

In making a study of the megaspores in some Carboniferous coals from Northern Ireland, I have found some objects evidently representing the megaspores of *B. fusiformis* (i.e. of *S. burntislandica*). Examination of these spores and those occurring in the petrified *Bensonites* suggests a new interpretation of its megaspore formation and of the dispersal mechanism.

The Spore Tetrads in Coal

The megaspores from Northern Ireland were obtained by maceration of coal. The coals concerned are of both Lower and Upper Carboniferous ages (Dinantian–Namurian) and come from the Ballycastle coalfield. The coals were broken down with a hammer until no fragments were larger than 1 cm., and placed in a saturated solution of potassium chlorate in concentrated nitric acid (Schulze's solution) for several days. The acid solution was then decanted

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

off, the coal washed, treated with dilute sodium hydroxide solution, and the residue further washed and separated into various size-grades by sieving. This procedure is a modified form of one of a number of methods of extracting fossil spores from coal (cf. Dijkstra, 1946).

The spore tetrads, described below as *D. scotti* gen. et sp. nov., occur among a variety of other forms—mostly lycopod megaspores of types known from the Scottish Namurian coals (Dijkstra, 1957). The specimens of *D. scotti* consist of two large spores (the pair being about 0·4 mm. long and 0·2 mm. wide) and two much smaller ones (about 40μ in diameter) all adhering together (Plate Nos. 1 and 2). These four spores evidently represent a highly modified tetrad, in which two spores became large (the 'fertile spores') and two remained minute (the 'abortive spores'). All four became sufficiently cutinized to survive fossilization in coal, and subsequent maceration. The tetrad is enclosed in a fine meshwork of cuticular substance which projects locally beyond the margin of the flattened fertile spores, and leaves a reticulate pattern where it passes over their surface.

Subsequent search among spores obtained from coals of comparable age from Berwickshire and Fife, Scotland, and Cumberland, England has revealed this same type of spore tetrad.

The development of a single megaspore tetrad into two fertile and two abortive members is unique among all known fossil spores. These spores are accordingly assigned to a new genus and species:

DIDYMOSPORITES gen. nov.

Diagnosis. Megaspores occurring as a tetrad composed of two large thin-walled fertile spores and two minute abortive ones, all enclosed within a loose cuticular network.

Type species. *D. scotti* gen. et sp. nov., from the Dinantian and Namurian of Britain (see below).

D. scotti *gen. et sp. nov.*

Diagnosis. Megaspore tetrad 360–582μ measured along the line of the two fertile spores (mean of 26 specimens, 449μ, standard deviation $d = 53\mu$); maximum width of tetrad at right-angles to this, 146–257μ (mean of 26 specimens, 205μ, $d = 27\mu$). Fertile spores formed of a thin membranous wall with numerous secondary folds (produced by crumpling on compression) and a fine irregular reticulate pattern formed by the loose cuticular network enclosing the tetrad. Two abortive spores present at the line of junction between the large fertile ones; flattened, more or less circular in outline, 31–63μ in diameter (mean of 26 specimens, 43μ, $d = 7\mu$).

Holotype (Plate No. 1; Fig. 1*a*). Specimen no. V 34641, Geology Department, British Museum, Natural History. From the coal seam under the Main Limestone, Gobb, County Antrim, Northern Ireland. Uppermost Dinantian (Lower Carboniferous).

Distribution. From coals of Namurian (Upper Carboniferous) and Dinantian (Lower Carboniferous) age in Britain.

Localities. D. scotti was obtained from coals from the following localities:

(1) Ballycastle Coalfield, Co. Antrim, Northern Ireland. The Splint Coal, Bath Lodge (Namurian); coal under the Main Limestone, Gobb (Dinantian); the Carrickmore Black Band, Carrickmore (Dinantian); the White Mine Coal, Murlough Bay (Dinantian). (The age of these coals is based on an oral communication from Dr. W. H. Ramsbottom.)

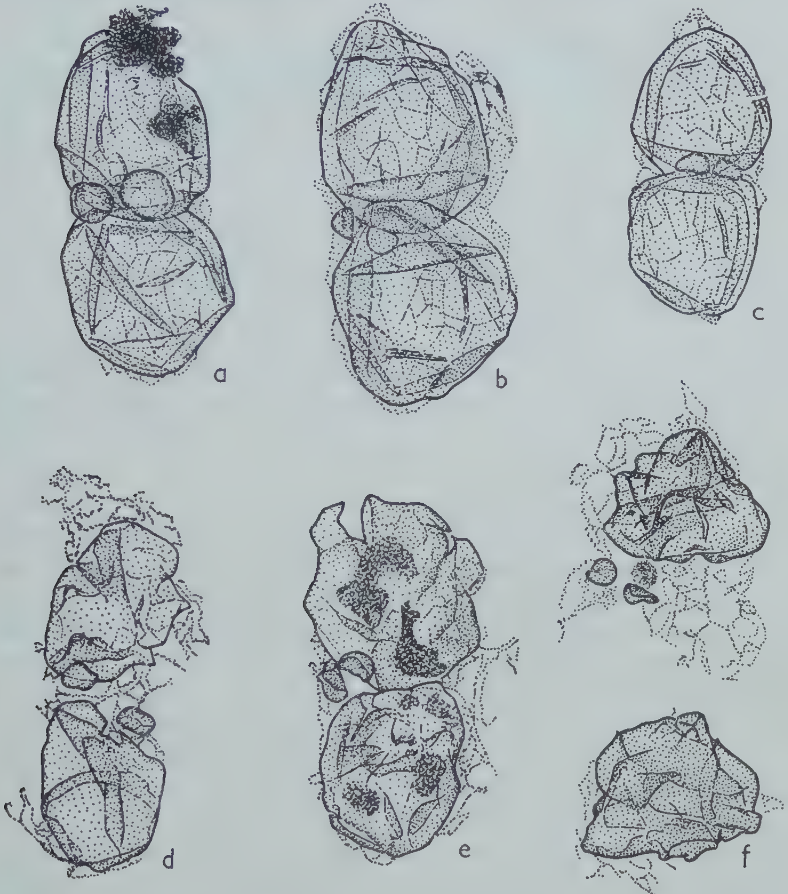


FIG. 1: *a*, *b*, and *c*. *Didymosporites scotti* gen. et sp. nov., × 100. Megaspore tetrads showing the two large fertile spores and the two minute abortive ones. Remains of the cuticular network are shown locally at the margin, and forming a reticulate pattern on the spore surface. *a* is the holotype; *a* and *b* are also shown on Plate Nos. 1 and 2. All from the coal under the Main Limestone, Gobb, N. Ireland. *d*, *e*, and *f*. Megaspores of *Bensonites fusiformis*, × 100, extracted from the Pettycur Limestone, showing the same features as the *Didymosporites*. *e* and *f* are also shown in Plate Nos. 4 and 5.
(Slides nos. V 34641–V 34645, British Museum, Natural History.)

(2) Fife, Scotland. Coal outcropping $\frac{1}{4}$ mile east of Pittenweem Harbour, about 900 ft. below the Hurlet Limestone; coal outcropping on the east

side of Billow Ness, Pittenweem Harbour, about 3,000 ft. below the Hurlet Limestone. Both Dinantian.

(3) Berwickshire, Scotland. Two coals outcropping in Cove Harbour, Cockburnspath. Calciferous Sandstone Series (Dinantian), probably equivalent to the coals of the Scremerston Group (see Pringle, 1948, p. 62, fig. 11).

(4) Cumberland, England. The Nether Hill seam, near Nether Hill Farm, and from a seam 170 ft. below Oakshaw Ford seam, near Dodgsontown Ford. Both from near Roadhead, Cumberland; Scremerston Coal Group, Dinantian.

COMPARISONS

The Megaspores contained in B. fusiformis

The megaspore tetrads described above show a striking similarity with those seen in sections of *B. fusiformis*, the megasporangia of *S. burntislandica*, as illustrated by Mrs. R. Scott (1908) and Surange (1952). The latter author investigated the megasporangia by means of a continuous series of peel sections, and showed them to consist of a swollen mass of parenchyma at the base, with two thin-walled megaspores in the distal part. Occasionally a third rather smaller spore appeared to be present.

A useful means of supplementing the information obtained by sectioning petrified sporangia is to dissolve the matrix to obtain the cutinized spores as whole, three-dimensional objects. This is especially useful for comparing spores in sporangia with those obtained by maceration of coal (Schopf, 1941, p. 17). A specimen of Pettycur Limestone containing abundant *Stauropteris* and *Bensonites* was accordingly dissolved in dilute hydrochloric acid, and the resulting sludge treated with Schulze's solution for about 2 hours. The residue contained many 'pairs' of *Stauropteris* megaspores, each still contained within a cuticular network, but otherwise free from sporangial tissue (Plate Nos. 3–5; Text-fig. 1*d–f*). The spore wall and other details were found to show most clearly on staining with saffranin and mounting in balsam.

The *Stauropteris* megaspores obtained in this way proved in nearly every case to have two very minute, abortive spores sited at the line of contact of the two large megaspores. Of the twelve specimens stained and mounted, ten showed these abortive spores; in the other two they appeared to be obscured by debris and the cuticular network. The contents of the megasporangium therefore evidently represent a single tetrad composed of two large fertile spores and two minute, abortive ones, all of which were cutinized. The fertile spores are very thin walled, and the wall is loosely convoluted or buckled (Plate No. 3). This same feature may be seen in some sections of *Bensonites* (cf. Plate No. 6); it also shows in Mrs. Scott's original figures. The degree of buckling varies, and it may have been caused by shrinkage of the spore contents during fossilization.

The whole tetrad is enclosed in a network of cuticular substance, resistant to Schulze's solution and staining in saffranin. This forms a thin layer surrounding the two fertile spores, passing between them and enmeshing the abortive spores. It appears to correspond with what Surange has called the 'spore bag' in his sectioned material. It probably represents the remains of a tapetum.

Comparison of Didymosporites scotti *and the Megaspores in* B. fusiformis

A comparison may now be made between the megaspore tetrads from coal described as *D. scotti* and those obtained from *B. fusiformis*. Both consist of the arrangement (unique among both living and fossil plants) of a single megaspore tetrad modified to form a pair of large fertile thin-walled megaspores and two minute abortive ones, all enclosed within a cuticular network. The dimensions of the two groups of spores (based on twelve specimens of *Bensonites* and twenty-six of *Didymosporites*) are as follows:

	Bensonites fusiformis megaspores			*Didymosporites scotti*		
	Range	Mean	Standard deviation	Range	Mean	Standard deviation
Length along the two fertile megaspores . .	404–467μ	*437μ*	21μ	360–582μ	*449μ*	53μ
Diameter of largest megaspore . . .	165–214μ	*189μ*	18μ	146–257μ	*205μ*	27μ
Diameter of abortive spores . . .	27–55μ	*42μ*	10μ	31–63μ	*43μ*	7μ

These data are consistent with the conclusion that *D. scotti* represents the isolated megaspore tetrad of *B. fusiformis*. The small differences in their measurements (3, 8, and 2 per cent. respectively, in the three dimensions given above) may reasonably be attributed to their different states of preservation. The only horizon from which *B. fusiformis* is known (the Pettycur Limestone, Dinantian) is within the range of the several records of *D. scotti* (Dinantian and Namurian), so that on age grounds their correlation is perfectly reasonable.

Although on all features of comparison the megaspores of *B. fusiformis* agree with *D. scotti*, these names cannot be regarded as synonyms. Spores isolated from coals are fossils of an entirely different category from the petrified sporangia on which *B. fusiformis* is based. Following current palaeobotanical practice separate names for these fossils must be retained.

DISCUSSION AND CONCLUSIONS

The two species of Stauropteris

Of the two species of *Stauropteris*, we know the petrified rachises, megasporangia, and now the megaspores of the Lower Carboniferous *S. burntislandica*, and the petrified rachises and microsporangia of the Upper Carboniferous *S. oldhamia*. Mrs. R. Scott (1908) attributed some microsporangia to the former species, but this was based only on association in one section. Proof that they really belong to *S. burntislandica* is still lacking. I have searched the numerous slides of *S. oldhamia* in the Scott collection for signs of megasporangia corresponding with *Bensonites* but have found none.

It is, of course, possible that *S. oldhamia* was homosporous as Scott supposed, while the seemingly similar *S. burntislandica* was heterosporous. The two species are included in the one genus on the basis of vegetative structure, and they may not be as closely related as this feature alone suggests.

The number of Megaspores in Bensonites

Surange regarded the *Bensonites* megasporangium as normally containing only two fertile megaspores, with an occasional third one. Lacey *et al.* (1957) have recently cited evidence of the formation of up to eight megaspores in *B. fusiformis*. This suggestion is based on a number of peel sections, separated by material lost in grinding. The deep buckling or convolutions of the *Bensonites* megaspores (Plate No. 3) frequently give an impression of a number of separate spores if seen in a single plane of section (Plate No. 6). It may be that the appearance of some of the sections of Lacey *et al.* were due to this effect. The *Bensonites* megaspores extracted from the Pettycur Limestone and described here suggest a regular formation of a complete tetrad with all four members cutinized, the two abortive spores being of very limited size. No more than these two large megaspores were seen in any instance.

The occurrence of a spore tetrad in which two members show a regular difference in development from the other two is obviously suggestive of a genetic mechanism. It would certainly be explicable in terms of segregation of some factor during the meiosis which presumably took place in tetrad formation. However, in *Lepidocarpon* the tetrad undergoes a comparable differentiation to form one large (fertile) and three small (abortive) megaspores. Such a 3:1 ratio in a haploid generation would be harder to explain on a genetic basis, and so far as I know this has never been suggested. It seems reasonable that whatever non-genetic mechanism controlled this process in *Lepidocarpon* (e.g. some physiological factor) could also have operated in *Bensonites*.

A further possibility which should be considered is that the two small spores in *Bensonites* might have functioned as microspores, the whole process of gametophyte formation and fertilization occurring within the sporangium. The recombination of the material segregated at meiosis, which would be implicit in such a process, would have similar genetic disadvantages to those associated with apomixis. But as the formation of mega- and microspores in the same sporangium is unknown in any living or fossil plant, such an arrangement seems in any case very unlikely. For this reason the two small spores in the *Bensonites* megasporangium are regarded as abortive megaspores comparable to those of *Lepidocarpon*.

Dispersal of the Bensonites *Megaspores*

Bensonites apparently lacks any kind of dehiscence mechanism in the sporangium wall, and Surange (p. 85) has suggested that 'the spores were liberated by rotting away of the cellular body'. The absence of any empty *Bensonites* megasporangia in the Pettycur Limestone at least bears out the point that the megaspores were not shed in a normal manner.

Walton (1952) and Martens (1956) have stressed the analogy between the prolonged apex of the *Bensonites* megasporangium and the receptive nucellar beak of some Pteridosperm seeds. Walton (1953) has further suggested that a cavity may have extended through the beak; if this were so, fertilization of archegonia within the undehisced megasporangium would be possible. Obviously, this is a very attractive hypothesis if one is to regard *Bensonites* as representing a morphological stage through which Pteridosperm ancestors might have passed in the evolution of the seed from the heterosporous condition.

The tetrads of *Didymosporites* occurring in coal offer a further piece of evidence in this direction. In all the coals in which they occur the two large fertile spores are always adhering in pairs. Now the free-sporing lycopod megaspores, very abundant in most Carboniferous coals, normally occur as individuals; rarely one finds a manifestly immature sporangial mass of lycopod megaspores, but these are far outnumbered by isolated megaspores of the same species. The only exception is *Lepidocarpon*; here the large, fertile spore (called *Cystosporites* Schopf) often occurs in coal with the three small, abortive spores adhering to its apex. In this case there is good evidence that the *Lepidocarpon* megasporangium became detached from the parent plant still enclosed within the 'integumented' megasporangium; it behaved, in this respect, like a seed.

The occurrence in coal maceration residues of *Bensonites* megaspores adhering as tetrads suggests that here, too, the whole megasporangium was regularly shed intact, still containing the mature megaspores. Only in this way would the megaspores always be in juxtaposition when compressed during coal-formation, and so adhere to one another. In retaining its megaspores within a megasporangium which was itself dispersed, *Bensonites* shows a significant analogy with both *Lepidocarpon* and true seed plants.

ACKNOWLEDGEMENTS

I am pleased to express my thanks to Dr. W. H. Ramsbottom and Mr. J. B. W. Day of the Geological Survey for help in obtaining coal samples, and to Professor T. M. Harris, F.R.S., Professor J. Walton, and Mr. P. R. Bell for helpful criticism. I am also grateful to Mr. R. Brinsden for taking the photographs.

LITERATURE CITED

DARRAH, W. C., 1941: The Coenopterid Ferns in American Coal Balls. Amer. Midl. Nat., **25** (2), 233–69.

DIJKSTRA, S. J., 1946: Eine monographische Bearbeitung der karbonischen Megasporen mit besonderer Berücksichtigung von Süd-limburg (Niederlande). Meded. geol. Sticht., Maastricht (C), **3**, 1. 1–101.

—— 1957. Lower Carboniferous Megaspores. Meded. geol. Sticht., Maastricht, N.S. **10**, 25–38.

LACEY, W., JOY, K. W., and WILLIS, A. J., 1957: Observations on the Aphlebiae and Megasporangia of *Stauropteris burntislandica* P. Bertrand. Ann. Bot., N.S., Lond., **21**, (84), 621–5.

MARTENS, P., 1956: Un Facteur évolutif négligé: le Bec nucellaire de l'ovule. Rev. gén. Bot., **63**, 529.

Pringle, J., 1948: British Regional Geology: The South of Scotland. 2nd ed. H.M.S.O., Edinburgh.

Schopf, J. M., 1941: Contributions to Pennsylvanian Paleobotany: *Mazocarpon oedipternum* sp. nov., and Sigillarian Relationships. Rep. Invest. Ill. geol. Surv., **75**, 1–40.

Scott, D. H., 1906: The Occurrence of Germinating Spores in *Stauropteris oldhamia*. New Phytol., **5**, (7), 170–2.

Scott, R., 1908: On *Bensonites fusiformis* sp. nov., a fossil associated with *Stauropteris burntislandica* P. Bertrand, and on the Sporangia of the latter. Ann. Bot., Lond., **22**, (88), 683–7.

Surange, K. R., 1952: The Morphology of *Stauropteris burntislandica* P. Bertrand and its megasporangium *Bensonites fusiformis* R. Scott. Phil. Trans., 237B 73–91.

Walton, J., 1952: L'Évolution des téguments et de la protection du sporange; in: Évolution et phylogénie chez les végétaux. Année Biol., 28 C 129.

—— 1953. The Evolution of the Ovule in the Pteridosperms. Adv. Sci., **10**, (38), 223–30.

EXPLANATION OF PLATE

(All the photographs are untouched, × 150.)

1. Holotype of *Didymosporites scotti* gen. et sp. nov.; megaspore tetrad, showing the two large, fertile spores and the two small, abortive ones.

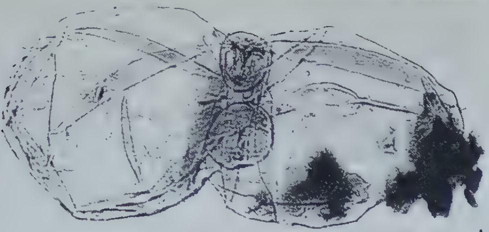
2. Another specimen, showing the reticulate pattern on the spore wall produced by the enclosing cuticular network.

3. Megaspores of *Bensonites fusiformis* extracted from Pettycur Limestone, showing deep folds of the spore wall and the enclosing cuticular network. (The abortive spores are closely pressed to the left-hand fertile spore.)

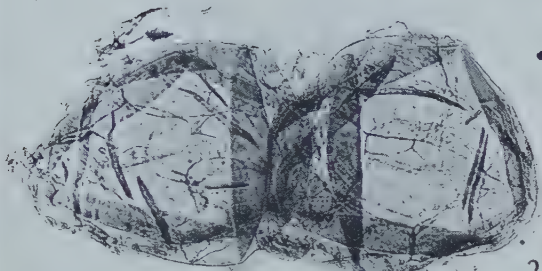
4 and 5. Spore tetrads of *B. fusiformis* from the Pettycur Limestone, showing the abortive spores (cf. Text-fig. 1*e* and *f*). In 5 the cuticular network has been ruptured by pulling the two fertile spores apart.

6. Peel section of *B. fusiformis* passing obliquely through the parenchymatous base of the megasporangium and tangentially through the two fertile megaspores, above; the uppermost megaspore at the margin of the photo has only just been cut into by the plane of section. The lower megaspore shows the folds of the spore wall, simulating a cluster of spores.

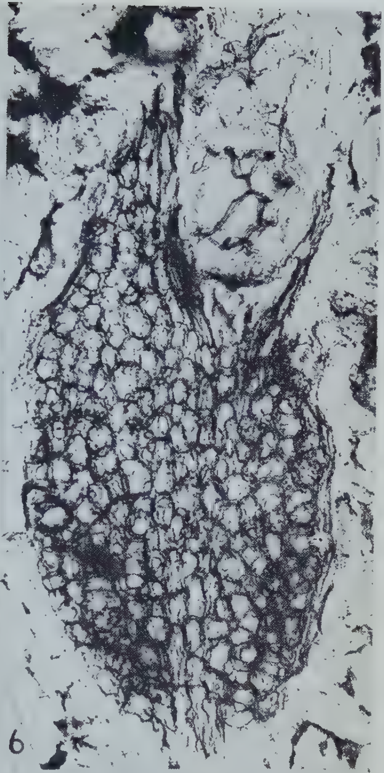
(Slides nos. V 34641, -42, -46, -44, -45, and V 21526 resp., British Museum, Natural History.)



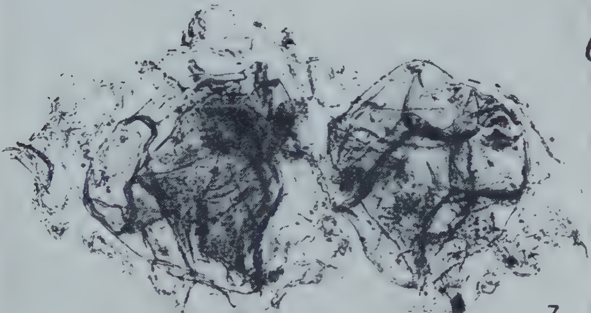
1



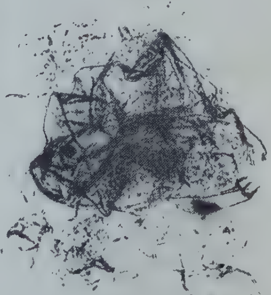
2

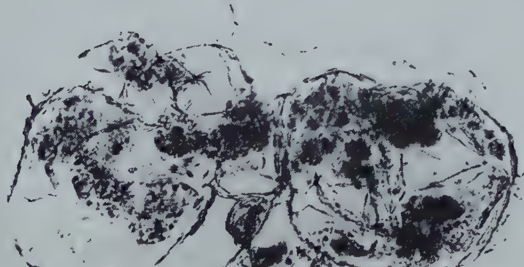


6



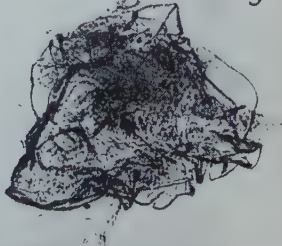
3





4

5



W. G. CHALONER

The cytology of *Halidrys siliquosa* (L) Lyngb.

BY

MARGARET NAYLOR

(*University of Hull*)

With four Plates

ABSTRACT

Resting nuclei in vegetative apices and developing receptacles of *Halidrys siliquosa* (L) Lyngb. contain several large, Feulgen-positive granules. The number of granules varies from two to nine in both vegetative cells and developing gametangia. Approximately 55 chromosomes have been counted in the meristoderm cells of the apical groove, and approximately 30 in the first, second, and third divisions of the antheridial initials.

METHODS

THE plants used in this investigation were collected from Wembury, Devon, and from the east coast of Yorkshire. Apices and receptacles were examined by Feulgen and acetocarmine squashes according to the following schedules.

Feulgen reaction. Small portions of material were fixed at room temperature for 12–18 hours either in acetic alcohol or in Karpechenko fixative. After thorough washing with distilled water, they were brought to a temperature of 60° C. in fresh, distilled water, hydrolysed at 60° C. in N.HCl for 15 minutes, and then returned to cold, distilled water to stop hydrolysis. They were next placed for 6–12 hours in freshly prepared Schiff's reagent (Manton, 1950, p. 295), bleached in three changes of SO_2 water, and examined by means of hand sections. These were squashed by firm pressure on the coverslip, the slides having been previously smeared with Haupt's adhesive. The coverslips were allowed to drop off into SO_2 water, and the preparations dehydrated, cleared, and mounted in Euparal.

After fixation with Karpechenko fluid, a good reaction was obtained using periods of hydrolysis varying from 15 to 45 minutes, a feature due (Hillary, 1939) to the chromic acid in the fixative. The failure to obtain a reaction with shorter periods of hydrolysis than 15 minutes might be due to incomplete removal of the formalin which may exert an inhibitory influence, but is more probably due to the resistance to penetration of the cell walls, as shorter hydrolysis times were also unsuccessful after acetic alcohol fixation.

This technique was used for developing vegetative cells and for developing antheridia, but has not yet been successfully applied to the oogonia of Halidrys, although it has succeeded in other genera. This failure is probably due to the lack of penetration of the reagents, and not, as suggested by Hillary (p. 295), to the failure to observe widely dispersed staining material in a large nucleus.

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

Acetocarmine squashes. Squashes were prepared according to the schedule already described (Naylor, 1957), and were successful for oogonia as well as for vegetative cells and developing antheridia.

SOMATIC NUCLEI

(*a*) *Resting nuclei.* The nuclei of the non-dividing somatic cells always gave an intense staining reaction both with acetocarmine and with Feulgen at whatever time of the year they were collected (cf. Moss and Elliot, 1957, p. 145).

These nuclei vary considerably in size according to the tissue in which they occur. Those in the cells of the meristoderm are the smallest with a diameter of about 3 μ (Plate 1 *e*). In the cortex the average diameter is about 4–5 μ, in the medulla about 10 μ or more (Plate 1 *a–d*).

The most conspicuous feature of these nuclei is the deeply staining granules of about 0·75–1·5 μ in diameter which they contain. The number of granules present is not constant, but 5 and 6 are the most frequently occurring numbers (Plate 1): as many as 9 and as few as 2 granules have been counted (Table 1).

TABLE 1

Variation in the Number of Granules in the Somatic Cells: Counts all made on Feulgen-stained Preparations

Type of tissue	Total number of nuclei counted	Nuclei with each of the following numbers of granules							
		2	3	4	5	6	7	8	9
Vegetative apex	113	1	11	13	30	31	24	2	1
Vegetative apex	129	1	11	24	36	41	12	3	1
Young receptacle	150	2	13	29	43	47	15	1	0
Totals	392	4	35	66	109	119	51	6	2

These granules give a very pronounced Feulgen reaction (Plate 1) and therefore differ in compositon from the nucleolus. In acetocarmine preparations the nucleolus is visible as an additional staining body, but is readily distinguished by its larger size and less intense staining reaction. Fine chromatin threads can also be distinguished in the nuclei. These are seen most clearly after the Feulgen reaction which also reveals numbers of much smaller granules distributed along the threads (Plate 1 *c*. Cf. Darlington and La Cour, 1941, Fig. 6).

The same structure is seen in the resting nuclei of the paraphyses.

(*b*) *Dividing nuclei.* These were most readily found in the region of the apical groove.

During prophase the nucleus enlarges slightly. The chromocentres thus become somewhat more widely separated, and since they also begin to lose their capacity to stain so intensely, the early prophase nuclei develop a curiously speckled appearance and give the tissue of actively dividing cells immediately behind the apex a very characteristic appearance. This feature is seen to an even greater extent in the developing antheridia. The chromosomes now differentiate as long, slender threads which contract and become shorter and thicker: by this stage the chromocentres are no longer distinguishable, though the nucleolus may still be visible. Further contraction reduces the chromosomes to short rods and finally, in late prophase, to almost spherical bodies evenly distributed throughout the nucleus (Plate 1 *h*, *j*), when very large numbers can be distinguished. The nucleolus and nuclear membrane then disappear.

The chromosomes arrange themselves into a compact metaphase plate and a pronounced intranuclear spindle is established. The diameter of the metaphase plate is considerably less than that of the prophase nucleus from which it develops (cf. Plate 1 *k–m*), and in polar view has a very regular appearance as the chromosomes are so contracted as to appear more or less circular. They are so crowded and small that unless the plate is absolutely flat an exact count is difficult. It is only possible to obtain a count at the metaphase stage as it is not preceded by a well-spread prometaphase in this species.

In side view the plate is flat and the chromosomes appear slightly elongated in the direction of the spindle (Plate 2 *a*).

Separation takes place initially as two regular, flat plates (Plate 2 *b–d*) which become curved at the end of anaphase. In telophase a closely packed clump of deeply staining material is formed before the resting stage is attained.

DEVELOPMENT OF THE ANTHERIDIA

Divisions in the antheridia were studied in material collected in January and February, by both acetocarmine and Feulgen techniques.

All stages of antheridial development from the differentiation of the antheridial initial to the mature antheridium could often be found in a single receptacle in basipetal sequence. Within any one conceptacle, however, all the antheridia were usually at more or less the same stage of development, and the initial division often appeared to be simultaneous throughout the conceptacle, the later divisions becoming a little staggered.

First division: In acetocarmine mounts the antheridial initials are readily distinguished by the homogeneous nature of the cytoplasm which becomes slightly staining with acetocarmine, contrasting with the non-staining cytoplasm of the vegetative cells. This affinity of reproductive initials for acetocarmine has also been remarked in *Stictyosiphon tortilis* (Naylor, in the press. Acta Adriatica). The nucleus is also very large relative to the size of the cell, which soon assumes a characteristic shape; these two features also allow ready recognition of the initials in Feulgen preparations where there is no staining of the cytoplasm to assist.

On its first differentiation, the nucleus of the antheridial initial passes into a resting stage and in all ways resembles a somatic nucleus—in size and in the presence of a conspicuous nucleolus plus about six or seven large chromocentres (Plate 2 *a*, *b*).

During the prophase of the first division the nucleus enlarges considerably to a diameter of about 8 μ, a far greater enlargement than in the vegetative cells.

Prophase is prolonged and in the early stages resembles that in the somatic cells, but in these larger nuclei the stages can be seen rather more clearly. The increase in size and the loss by the chromocentres of the capacity to take up stain again combine to give the early prophase the characteristic speckled appearance already described. The chromosomes begin to differentiate before the chromocentres have entirely disappeared, and often during the early prophase stages these can be clearly seen arranged at intervals along the threads (Plate 2 *c–e*). Gradually the chromosomes become more obvious and by the time they are fully differentiated as long, coiled threads the chromocentres can no longer be distinguished (Plate 2 *g*).

When they first differentiate, the chromosomes are long and slender and sometimes show traces of spiral coiling (Plate 2 *f*); they frequently show polarization (Plate 2 *g*, *h*), as in the zygotene stage of meiotic prophase and pairing is also seen.

During the later stages of prophase drastic contraction of the chromosomes occurs until they are more or less spherical. The nucleolus and the nuclear membrane disappear, an intranuclear spindle is organized, and the chromosomes arrange themselves on it in a regular, flat plate.

The metaphase plate is very crowded and compact, only about 4μ in diameter and the chromosomes so contracted as to appear more or less circular in polar view (Plate 2 *j–l*); in side view they again appear elongated in the direction of the spindle (Plate 3 *a*).

During anaphase two regular, flat plates separate (Plate 3 *b*) and at opposite ends of the cell curve and crowd together to form small, dense clumps before losing their capacity to stain and passing into a resting stage.

Second division: Only a brief interphase occurs before the second division which takes place without any cleavage of the cytoplasm. During this phase the chromocentres reappear, again losing their staining capacity as the two nuclei pass into prophase. Differentiation and contraction of the chromosomes follow and compact metaphase plates are organized (Plate 3 *e*, *f*): anaphase and telophase follow the usual course.

Further divisions: Usually four more divisions follow with the final production of 64 nuclei, the divisions taking place simultaneously in all the nuclei of the antheridium so that 4, 8, 16, and 32 metaphase plates are seen at successive divisions (Plate 3 *g*, *j*, *k*). No cleavage of the cytoplasm appears to take place until the final division is completed.

Between successive divisions there are brief resting phases during which the chromocentres are visible. During these divisions the chromocentres are rather smaller than in the somatic cells and less deeply staining (Plate 3 *h*); they are also smaller relative to the size of the nuclei as a whole, which almost fill the entire antheridium, and which otherwise appear clear and completely non-staining. Thus, the developing antheridium as a whole has a characteristic pale and speckled appearance during the resting stages, in striking contrast to the stages in division when numerous compact and deeply staining metaphase plates and anaphases with clumped chromosomes are seen. The number of chromocentres varies, but seems to show the same range of variation as seen in the somatic cells (Table 2).

After the final division the nucleus of the sperm passes into the resting stage. It is about 2 μ in diameter and contains a number (usually 4 or 5) of relatively large and intensely staining chromocentres. In this condition the sperms are liberated (Plate 3 *l*).

TABLE 2

Variation in the Number of Granules in the Nuclei of Developing Antheridia: all Counts made on Feulgen-Stained Preparations

Stage of development of the antheridium	Total number of nuclei counted	Nuclei with each of the following numbers of granules							
		2	3	4	5	6	7	8	9
1-nucleate	76	1	1	11	26	23	10	4	0
2-nucleate	29	0	2	2	9	8	5	3	0
4-nucleate	7	0	2	0	2	1	2	0	0
8-nucleate	10	0	0	4	4	0	2	0	0
16-nucleate	53	0	0	3	24	22	4	0	0
32-nucleate	14	0	0	3	6	5	0	0	0
64-nucleate	44	0	2	8	19	11	3	1	0
Total	233	1	7	31	90	70	26	8	0

DEVELOPMENT OF THE OOGONIA

Dividing nuclei were found in material collected at Filey, Yorkshire in early and mid-February (2nd and 16th), the former occasion being a mild sunny morning with a cool breeze, the latter a bright sunny morning after a fall of snow (cf. Moss and Elliot, 1957, p. 147).

First division: Before the first division the nucleus undergoes a resting phase lasting several months. The prophase of the first division may also be prolonged, the preliminary enlargement being seen in material collected in January.

During prophase the nucleus enlarges to a diameter of about 17–25 μ: the nucleolus enlarges correspondingly to a diameter of 6–7 μ. Usually during this phase of enlargement the contents of the nucleus appear clear and non-staining, but a number of deeply staining granules, similar in size to those in the vegetative nuclei, may be present. Probably the chromocentres usually lose their capacity to stain during this prolonged resting stage, which precedes this first division, a view which is supported by their behaviour during the second and third oogonial divisions.

During this phase of enlargement a further spherical or discoid body, staining with acetocarmine, is sometimes seen close to the nucleolus (Plate 4 *b*). This is probably a 'chromophilous spherule' such as is recorded in the oogonia of *Coccophora langsdorfii* (Tahara, 1929), *Sargassum horneri* (Okabe, 1929), and *Carpophyllum flexuosum* (Dawson, 1940); in the antheridia of *Hizikia fusiformis* (Inoh and Hiroe, 1954 *b*), *Sargassum piluliferum* (Inoh and Hiroe, 1954 *c*), and *Sargassum tortile* (Inoh and Hiroe, 1956); and as a 'staining body associated with the nucleolus' in the oogonia of *Fucus vesiculosus* (Yamanouchi, 1909, Fig. 44*a*). In *H. siliquosa* this persists throughout the division and although it was not seen to divide, a similar body appeared in each of the daughter nuclei during the second prophase and in each of the four resulting nuclei at the third prophase (Plate 4 *g*).

After completion of the enlargement differentiation of the chromosomes begins. They first appear as slender, coiled threads evenly distributed throughout the nucleus (Plate 4 *c*), but as they condense they become localized in their distribution and form a small peripheral cap (Plate 4 *d*). During this phase the nucleolus gradually loses its capacity to take up stain. When fully contracted the chromosomes, as in the vegetative and antheridial cells, are almost spherical.

Despite the large size of the prophase nucleus, the metaphase plate has about the same diameter (approximately 5 μ) as at the first division of the antheridial initial (Plate 4 *e*). A spindle is organized occupying only a small proportion of the nucleus and often asymmetrically placed: the 'chromophilous spherule' is often visible at this stage lying close to the spindle midway between the equatorial plane and one of the poles: it is usually spherical at this stage. The matrix of the nucleus at this stage becomes finely granular and rather deeply staining, and the nuclear membrane disappears.

Only the beginning of anaphase has been seen, when the chromosomes separate as two flat plates. During telophase the chromosomes form two closely clumped, densely staining masses before passing into the resting stage.

Further divisions: The two daughter nuclei resulting from this division are, at their first appearance, small and contain deeply staining chromocentres. They rapidly pass into the prophase of the second division, enlarging to a diameter of approximately 10 μ, losing their staining granules and becoming quite clear and non-staining in appearance. Two small metaphase plates are organized, still within the boundaries of the original nucleus. Each has a conspicuous spindle with an associated 'chromophilous spherule' as in the first division (Plate 4 *f*). Anaphase with separation of flat plates, and telophase with clumping of the chromosomes follow, and four daughter nuclei are formed.

These nuclei are at first small and occupy the space of the original nucleus and contain chromocentres. Like the nuclei from the previous division, they rapidly enlarge to a diameter of about 10 μ (Plate 4 *j*) and become clear and non-staining, separating and dispersing throughout the cytoplasm in the process.

According to Moss and Elliot (1957, p. 147) only four nuclei are formed, one becoming the egg nucleus, the remainder being extruded. However, in the material from Filey a third division consistently occurred giving rise to eight nuclei. Four small metaphase plates are formed, each with a conspicuous spindle and associated 'chromophilous spherule', as in the two previous divisions. Anaphase and telophase follow in the characteristic manner.

The eight daughter nuclei are at first small and contain chromocentres (Plate 4 *k*). Seven of these nuclei are extruded from the cytoplasm. These supernumerary nuclei retain their chromocentres and lie between the cytoplasm and oogonium wall as curved, flattened disks. The eighth nucleus assumes a central position, enlarges to a diameter of about 15 μ, loses its chromocentres and passes into prophase with differentiation of long, slender chromosomes.

Typically the mature egg nucleus is surrounded by a region of cytoplasm differentiated from the bulk of the oogonial cytoplasm which contains large, clear and lightly staining granules of reserve food materials. In contrast to this, the region around the nucleus is finely granular, densely staining with acetocarmine and stellate in outline (Plate 4 *l*, *m*). A similar region has been observed surrounding the mature egg nucleus in other fucoids, viz: *Carpophyllum flexuosum* (Dawson, 1940) and in *Cystoseira barbata* (Nienburg, 1910).

There are thus two striking differences between the details of nuclear division in the antheridia and in the oogonia. In the former, differentiation of the chromosomes begins whilst the chromocentres are still visible: in the oogonia the chromocentres completely lose their capacity for staining before any differentiation of chromosomes occurs. Second, the 'chromophilous spherule' seen in the oogonia is not recorded in the antheridia.

CHROMOSOME COUNTS

Chromosome counts were made from photographic enlargements of metaphase plates. These were enlarged by 10,000 diameters, the chromosomes outlined and visual checks then made against the nuclei. Only a few counts were made and usually only approximate counts were obtained owing to the plates not being completely flat.

Somatic cells. Only two counts were made, both on nuclei of meristoderm cells within the apical groove. Both gave counts of 55 plus a number of chromosomes lying above or below the plate which could not be counted with certainty. One plate is illustrated in Plate 1 *k–m*.

Antheridia. A number of counts were made at the metaphase of the first division and two at the metaphase of the second division. The figures varied between 30 and 34. Some of the nuclei on which counts were made are shown in Plates 2 *j–l* and 3 *e*.

Oogonia. Only one count of approximately 28 was made on a metaphase plate at the first division of the oogonial initial. The nucleus is illustrated in Plate 4 *e*.

DISCUSSION

According to Moss and Elliot (1957, p. 145) the resting nuclei of *Halidrys siliquosa* are small and generally possess a large and conspicuous nucleolus. In the present investigation such a condition was not seen; the resting nuclei possessed several relatively large, densely staining chromatin granules which could be distinguished from the nucleolus by their pronounced Feulgen reaction. These granules were not described by Moss and Elliot from their gentian violet and Heidenhain's haematoxylin preparations, and they did not apply the Feulgen reaction to their material. It is, however, possible that the densely staining particles which they describe in the prophase of the vegetative and antheridial nuclei, and the refractive granules in the oogonial nuclei may correspond to them.

The granular condition of the nuclei of *Halidrys siliquosa* was first described and illustrated by le Touzé (1912, p. 34 and Plate 9, Fig. 1), and later by Roy (1938, p. 162 and Pl. 8), who demonstrated the granules using a variety of fixatives and stains, including Feulgen and Heidenhain's haematoxylin. The number of granules was said to vary from 4 to 7 (le Touzé) or from 3 to 7 (Roy).

The illustrations of Doubt (1928) indicate that the nuclei of *Halidrys dioica* also have a granular structure, and I have now been able to confirm that this is the case.[1] Granular nuclei thus appear to be characteristic of the genus.

Feulgen positive granules in the resting nuclei of angiosperms are known to be of two kinds, prochromosomes and chromocentres. Prochromosomes are peripheral in position and show an exact numerical correspondence with the chromosomes of which they represent a particular portion (Manton, 1935). As the granules in the nuclei of *Halidrys siliquosa* vary in number and bear no relationship to the number of chromosomes, it is unlikely that they are prochromosomes, as suggested by Roy (p. 132).

Chromocentres are distributed throughout the nucleus as portions of the chromatin reticulum, and may vary in number from cell to cell. Darlington and La Cour (1941) attribute this variation in number to the condensation of the chromatin threads and the consequent fusion of the granules, contraction and fusion being greatest in the smallest nuclei. In *Halidrys siliquosa* the number of granules is, on the whole, greater in the large nuclei of the medullary cells than in the small nuclei of the meristoderm cells (cf. Plate 1 *a*, *b*, *e*). Darlington and La Cour also showed that the number of granules was not related to the degree of ploidy of the nucleus. In a single plant of *Fritillaria pudica* they found 23 chromocentres in an embryo sac mother cell, 21 in a reduced pollen grain, and 21 in a cell of a triploid root tip. A similar lack of correlation between granule number and chromosome complement is seen in *Halidrys siliquosa* where the same range of numbers of granules is seen in both the (diploid) vegetative cells and the (haploid) cells of the developing antheridia (Table 2). These comparisons suggest that the granules in the resting nuclei of *Halidrys siliquosa* are probably chromocentres. There is a close comparison between the Feulgen-stained medullary cell nucleus of *Halidrys siliquosa* illustrated in Plate 1 *c* and that of the embryo-sac mother-cell illustrated by Darlington and La Cour (1941, Fig. 6).

The chromocentres of *Halidrys siliquosa* disappeared during prophase, when they were clearly seen to be arranged at intervals along the developing chromosomes, but extreme condensation made it impossible to determine whether they were represented at metaphase by unstained portions of the chromosomes as described by Darlington and La Cour (1940) in *Trillium*.

---

[1] I wish to thank Professor E. Yale Dawson for the material used in this investigation.

Le Touzé suggested that granular nuclei of this type were characteristic of the Cystoseiraceae, and Roy showed that they were also found in *Bifurcaria rotunda* (Huds.) Papenfuss (sub *B. tuberculata* Stack.) and in *C. tamariscifolia* (Huds.) Papenfuss (sub *C. ericoides* (L) Ag.) but not in *C. granulata* Ag., *C. foeniculacea* (L) Grev., and *C. myriophylloides* (Sauv.). I have been able to confirm these observations and to extend them to further species of Cystoseira. Large Feulgen-positive granules are also found in the nuclei of *C. corniculata* Hauck, *C. spicata* Ercegović and *C. spicata* var. *elegans* Ercegović, but not in seven other species of Cystoseira which were examined. Possibly the chromatin granules described and figured by Nienburg (1910) in developing oogonia of *C. barbata* are also chromocentres. Thus, although granular nuclei are not uncommon in the Cystoseiraceae, their occurrence is not so consistent as originally suggested by le Touzé.

TABLE 3

Chromosome Counts for Members of the Fucales

	Antheridium	Oogonium	Germling	Method	Author
FUCACEAE					
Fucus vesiculosus	32	32	64	Heidenhain and Flemming's safranin	Yamanouchi, 1909
Fucus evanescens	—	32	—	Heidenhain	Inoh, 1935
Pelvetia wrightii	—	32	—	Heidenhain	Inoh, 1935
CYSTOSEIRACEAE					
Coccophora langsdorfii	—	32	—	Heidenhain	Tahara, 1929
	—	—	64	—	Tomita, 1932
Cystophyllum crassipes	—	—	64	Heidenhain	Inoh, 1944
Cystophyllum sisymbrioides	—	32	—	—	Shimotomai, 1928
SARGASSACEAE					
Hizikia fusiformis	32	—	—	—	Inoh and Hiroe, 1954*b*
Sargassum confusum	32	—	—	—	Abe, 1933
Sargassum enerve	—	30 approx.	—	—	Tahara and Shimotomai, 1926
Sargassum horneri	16	16	32	Heidenhain	Kunieda, 1928
	—	32	—	Heidenhain	Okabe, 1929
	—	—	64	—	Okabe, 1930
	32	—	—	—	Inoh and Hiroe, 1954*d*
Sargassum patens	—	—	64	—	Inoh and Hiroe, 1954*e*
Sargassum piluliferum	32	—	—	—	Inoh and Hiroe, 1954*c*
	—	—	64	—	Inoh and Hiroe, 1954*a*
Sargassum tortile	32	—	—	—	Inoh and Hiroe, 1956

Yamanouchi (1909) followed nuclear behaviour in *Fucus vesiculosus* and counted 64 chromosomes in the nuclei of vegetative cells and 32 at the first and subsequent divisions of developing antheridia and oogonia, showing that the thallus was diploid and that meiosis occurred in the developing gametangia. This appears to be true for the other members of the Fucales which have been investigated so far, and in most cases the same basic number ($n = 32$)

has been reported (Table 3). Deviations from this figure are reported only by Osborn (1949) who found 24 chromosomes in the vegetative cells of *Hormosira banksii* and 12 in the developing antheridia, and by Moss and Elliot (1957) who record a basic number of $n = 8$ for *Halidrys siliquosa*. Moss and Elliot also record 8 chromosomes at the first divisions of the antheridia and oogonia and suggest that the thallus is haploid and that meiosis takes place on the germination of the zygote.

In the present investigation it has only been possible to make a small number of chromosome counts, but the figures obtained (55 in the vegetative cells, approximately 30 in the first and subsequent divisions of the antheridia, and approximately 28 in the first division of the oogonia) are of the order of those obtained by Yamanouchi, and suggest that the thallus is diploid with meiosis taking place in the developing gametangia.

Meiotic division in the antheridium is further indicated by the sequence of stages in the prolonged prophase: in the oogonium the evidence is more scanty, but similar stages are seen. The 'chromophilous spherule' seen in the divisions within the oogonia was first described by Williams (1904) in the tetrasporangia of *Dictyota* and later (Georgevitch, 1918; Carter, 1927) in *Padina pavonia* and is peculiar to the Phaeophyta. In all instances hitherto recorded (p. 209) it has only been seen accompanying a meiotic division. It is not mentioned by Moss and Elliot.

Comparison of Moss and Elliot's Figs. 1B, 2B, 3B, and 5B with Plates 1 *d–f*, 2 *b*, and 4 *a* in this paper respectively, indicate that the eight chromosomes counted by these workers correspond to the Feulgen-positive bodies of the present investigation. The distinctive shapes recorded by Moss and Elliot may be the result of the embedding and sectioning technique they employed, whereas the rounded contours seen in the present investigation—except during prophase when the chromocentres often appear angular—may be the result of the acetocarmine and Feulgen techniques used, as both tend to cause swelling.

The present investigation thus indicates that *Halidrys siliquosa* conforms to the usual condition in the Fucales of a diploid thallus with a large number of chromosomes and with meiosis occurring at gamete formation, and also confirms the observations of le Touzé and Roy on the granular structure of the resting nucleus.

SUMMARY

1. Resting nuclei of somatic cells, paraphyses, developing antheridia and oogonia of *Halidrys siliquosa* contain several relatively large chromocentres.

2. Dividing nuclei of vegetative cells in the apical groove show a large number of chromosomes at metaphase: approximately 55 have been counted.

3. During the development of the antheridia six successive divisions produce 64 sperms. At the metaphase of the first division—and at any later divisions which have been counted—there are approximately 30 chromosomes.

4. Three divisions resulting in the production of eight nuclei occur in developing oogonia: seven of these nuclei are extruded from the cytoplasm. Approximately 28 chromosomes have been counted at metaphase of the first division.

5. A chromophilous spherule is associated with the nucleolus and spindle in all the divisions in the oogonium.

6. During prophase in the developing antheridia the chromosomes differentiate whilst the chromocentres are still distinguishable: in the developing oogonia the chromocentres disappear completely before the chromosomes begin to differentiate.

7. The mature sperms are liberated with their nuclei in the resting condition with prominent chromocentres: in the mature eggs the nuclei are in prophase with long, slender chromosomes already differentiated.

LITERATURE CITED

ABE, K., 1933: Mitosen in Antheridium von *Sargassum confusum*. Sci. Rep., Tôhoku Imp. Univ. 4. Ser., **8**, 259.

CARTER, P. W., 1927: Life History of *Padina pavonia*. Ann. Bot., **41**, 139.

DARLINGTON, C. D., and LA COUR, L. F., 1940: Nucleic Acid Starvation of Chromosomes in *Trillium*. J. Genetics, **40**, 185.

—— —— 1941: The Detection of Inert Genes. J. Hered., **22**, 115.

DAWSON, A. E. E., 1940: Studies in the Fucales of New Zealand. 2. Observations on the Female Frond of *Carpophyllum flexuosum* (Esp.) Grev. New Phytol., **39**, 283.

DOUBT, D. G., 1928: Cytology of *Halidrys dioica*. Bot. Gaz., **86**, 330.

GEORGEVITCH, M. P., 1918: Génération asexuée du *Padina pavonia*. C. R. Acad. Sci., Paris, **167**, 595.

HILLARY, B. B., 1939: The use of the Feulgen Reaction in Cytology. Bot. Gaz., **101**, 276.

INOH, S., 1935: Embryological Studies on *Pelvetia wrightii* Yendo and *Fucus evanescens* Ag. J. Fac. Sci. Hokkaido Univ., 5 Ser., **5**, 9.

—— 1944: Embryological Studies on *Turbinaria* and *Cystophyllum*. Ibid., **5**, 199.

—— and HIROE, M., 1954*a*: Cytological Studies on the Fucaceous plants, I. On the Somatic Mitosis in the Embryo of *Sargassum piluliferum* C. Ag. La Kromosomo, **21**, 760.

—— —— 1954*b*: Ibid., II. On the Meiotic Division in the Antheridium of *Hizikia fusiformis* Okamura. Ibid., 764.

—— —— 1954*c*: Ibid. III. On the Meiotic Division in the Antheridium of *Sargassum piluliferum* C. Ag. Ibid., 767.

—— —— 1954*d*: Ibid. IV. On the Meiotic Division in the Antheridium of *Sargassum horneri* (Turn.) Ag. Bot. Mag., **67**, 190.

—— —— 1954*e*: Ibid. V. On the Mitotic Division in the Embryo of *Sargassum patens* C. Ag. Biol. J. Okayama Univ., **2**, 1.

—— —— 1956: Ibid. VI. On the Meiotic Division in the Antheridium of *Sargassum tortile* C. Ag. La Kromosomo, **27–28**, 942.

KUNIEDA, H., 1928: Development of the Sexual Organs and Embryogeny in *Sargassum horneri*. J. Coll. Agric. Tokyo, **9**, 383.

LE TOUZÉ, M. H., 1912: Contribution à l'étude histologique des Fucacées. Rev. gén. Bot., **24**, 33.

MANTON, I., 1935: Some New Evidence on the Physical Nature of Plant Nuclei from Intraspecific Polyploids. Proc. Roy. Soc. B, **118**, 522.

—— 1950: Problems of Cytology and Evolution in the Pteridophyta. *Cambridge*.

MOSS, B. L., and ELLIOT, E., 1957: Observations on the cytology of *Halidrys siliquosa* (L.) Lyngb. Ann. Bot., N.S., Lond., **21**, 143.

NAYLOR, M., 1957: An acetocarmine squash technique for the Fucales. Nature, **180**, 46.

NAYLOR, M., in the press: Some Aspects of the Life History and Cytology of *Stictyosiphon tortilis* (Rupr.) Reinke. Acta Adriatica.

NIENBURG, W., 1910: Die Oogonentwicklung bei *Cystoseira* und *Sargassum*. Flora, **101,** 167.

OKABE, S., 1929: Meiosis im Oogonium von *Sargassum horneri* (Turn.) Ag. Sci. Rep., Tôhoku Imp. Univ. 4 Ser., **4,** 661.

—— 1930: Mitosen im keimenden Embryo von *Sargassum horneri*. Ibid., 757.

OSBORN, J. E. M., 1949: The Structure and Life History of *Hormosira banksii* (Turn.) Decaisne. Trans. Roy. Soc. N.Z., **77,** 47.

ROY, K., 1938: Recherches sur la structure du noyau quiescent et sur les mitoses somatiques de quelques Fucacées. Rev. Algol., **11,** 101.

SHIMOTOMAI, N., 1928: Karyokinese im Oogonium von *Cystophyllum sisymbrioides* J. Ag. Sci. Rep., Tôhoku Imp. Univ. 4 Ser., **3,** 577.

TAHARA, M., 1929: Ovogenesis in *Coccophora langsdorfii* (Turn.) Grev. Ibid., **4,** 551.

—— and SHIMOTOMAI, N., 1926: Mitosen bei *Sargassum*. Ibid., **1,** 189.

TOMITA, K., 1932: Befruchtung und Kernteilung bei *Coccophora langsdorfii*. Ibid., **7,** 43.

WILLIAMS, J. L., 1904: Studies in the Dictyotaceae: I. The cytology of the tetrasporangium and germinating tetraspore. Ann. Bot., **18,** 141.

YAMANOUCHI, S., 1909: Mitosis in *Fucus*. Bot. Gaz. **47,** 173.

EXPLANATION OF PLATES

PLATE 1

Photomicrographs of vegetative nuclei of *Halidrys siliquosa*, all except (*g*) to the same scale, × 3,000. (*g*) × 1,500. In each case the line indicates a length of 10 μ.

(*a*), (*b*) Acetocarmine preparations of medullary cell nuclei, showing the nucleolus with 7 and 6 granules respectively. (*c*) Feulgen preparation of a medullary cell nucleus showing 5 large chromatin granules, several smaller ones, and chromatin threads. (*d*) Acetocarmine preparation of several cortical cell nuclei showing the smaller size and varying number of granules. (*e*) Feulgen preparation of meristoderm cell nucleus showing 5 granules. (*f*) Feulgen preparation of nucleus of a paraphysis showing 7 granules. (*g*) Feulgen preparation of part of the outer cortex and the meristoderm showing the granular nuclei. (*h*), (*j*) Two optical planes of a nucleus of a meristoderm cell in late prophase, showing a large number of small, contracted chromosomes. In (*h*) the nucleolus can be seen. Note increase in volume compared with the resting nucleus shown in (*e*). Acetocarmine preparation. (*k*), (*l*), (*m*) Three planes of a metaphase plate in a meristoderm cell, stained with acetocarmine and showing large numbers of small, closely arranged chromosomes. Analysis of this plate at an enlargement of 10,000 diameters gave a count of 55 chromosomes plus a number more in a higher plane which could not be clearly distinguished.

PLATE 2

Photomicrographs of the first division in the antheridial initial of *Halidrys siliquosa*, all to the same scale, × 3,000. In each case the line represents a length of 10 μ.

(*a*), (*b*) Resting nuclei of antheridial initials, (*a*) stained with acetocarmine and showing the nucleolus and 3 granules, (*b*) stained with Feulgen and showing 5 granules. (*c*), (*d*), (*e*) Early prophase stages showing the great increase in volume, (*c*) Feulgen preparation showing 3 large and several small granules on the differentiating chromatin threads. (*d*), (*e*) Two optical planes of the same nucleus, stained with acetocarmine, and showing the nucleolus and about 6 granules on the differentiating chromatin threads. (*f*) Later prophase in an acetocarmine preparation, showing the nucleolus and only 1 granule. The chromosomes have condensed to fairly stout threads and some show spiral coiling. (*g*) Later prophase with chromosomes well differentiated and no granules discernible. The nucleolus is still visible and the chromosomes show slight polarization. Acetocarmine. (*h*) 'Bouquet' stage in an acetocarmine preparation, showing pronounced polarization of the condensing chromosomes. (*j*), (*k*), (*l*) Polar views of 3 different metaphase plates on which counts have been made. (*j*), (*k*) Feulgen preparations, giving counts of 34 and 32 respectively. (*l*) Acetocarmine preparation, giving a count of 30.

PLATE 3

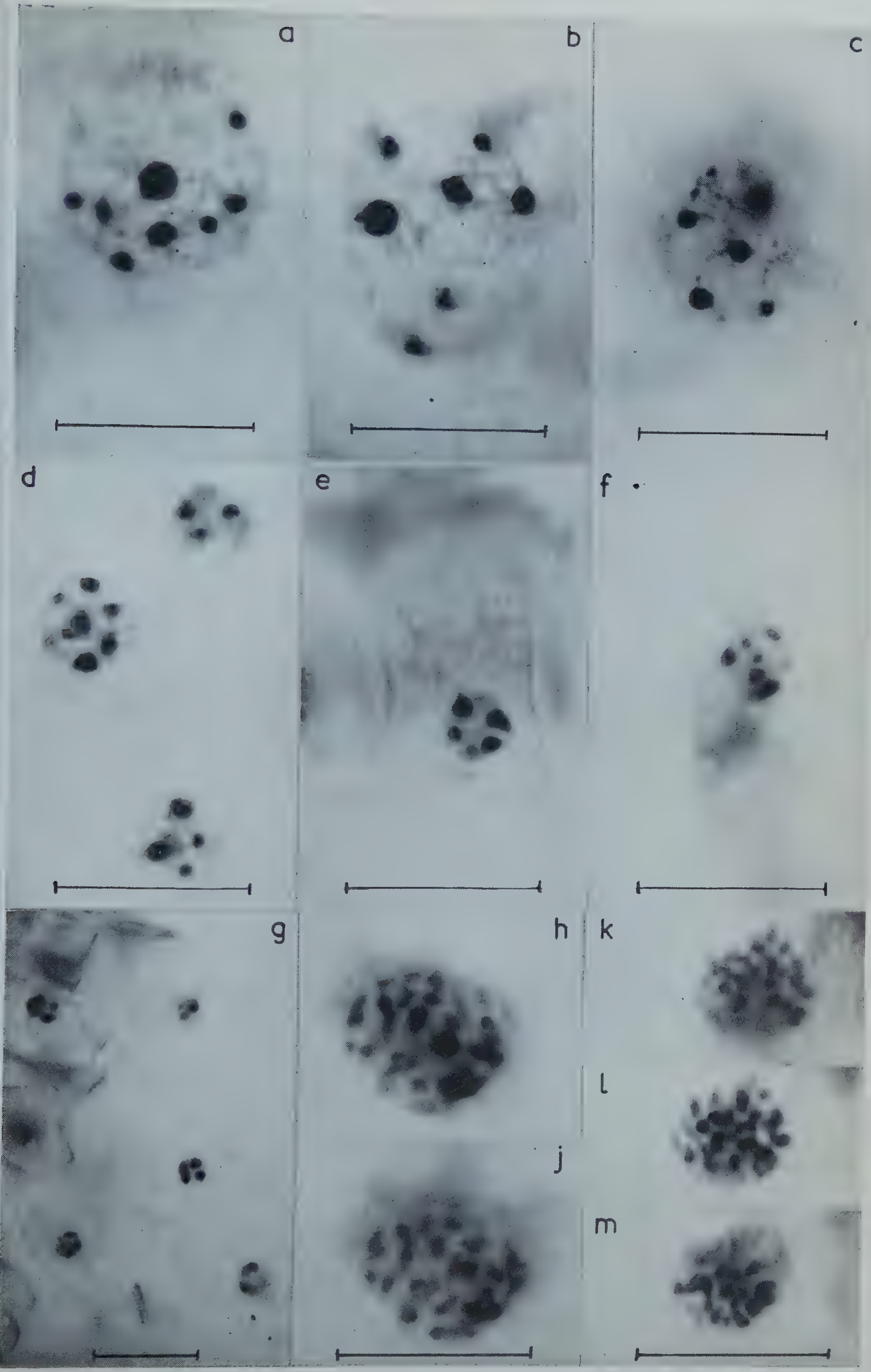
Photomicrographs of later stages in the development of the antheridium of *Halidrys siliquosa*, all to the same scale, × 3,000. In each case the line represents a length of 10 μ.

(*a*) Side view of the metaphase plate of the first division showing the spindle, the elongation of the chromosomes in the direction of the spindle, and the equatorial constriction as they separate. Acetocarmine. (*b*) Acetocarmine preparation of early anaphase of the first division, seen in side view and showing the separation as two flat plates. (*c*), (*d*) Two optical planes of the same cell showing polar views of the anaphase plates. Acetocarmine. (*e*) Metaphase of the second division, showing one plate in polar view and one in side view. An analysis of the plate in polar view gave a count of 30 chromosomes. Acetocarmine. (*f*) Side view of a metaphase plate of the second division showing the spindle and the elongation of the chromosomes in the direction of the spindle. Acetocarmine. (*g*) The third division showing 4 metaphase plates, 2 in polar view, 2 in side view. Acetocarmine. (*h*) Four-nucleate stage of the antheridium, showing the granules in the resting nuclei. Feulgen. (*j*) Fourth division showing 8 metaphase plates, 1 of which is in polar view. In the portion of a second antheridium seen in the top right-hand corner, the 16 nuclei are in the resting stage and their granular structure is visible. Acetocarmine. (*k*) Fifth division showing several of the 16 metaphase plates, 1 in polar view. Acetocarmine. (*l*) The nuclei of a group of sperms just liberated from an antheridium showing a varying number of granules. Acetocarmine.

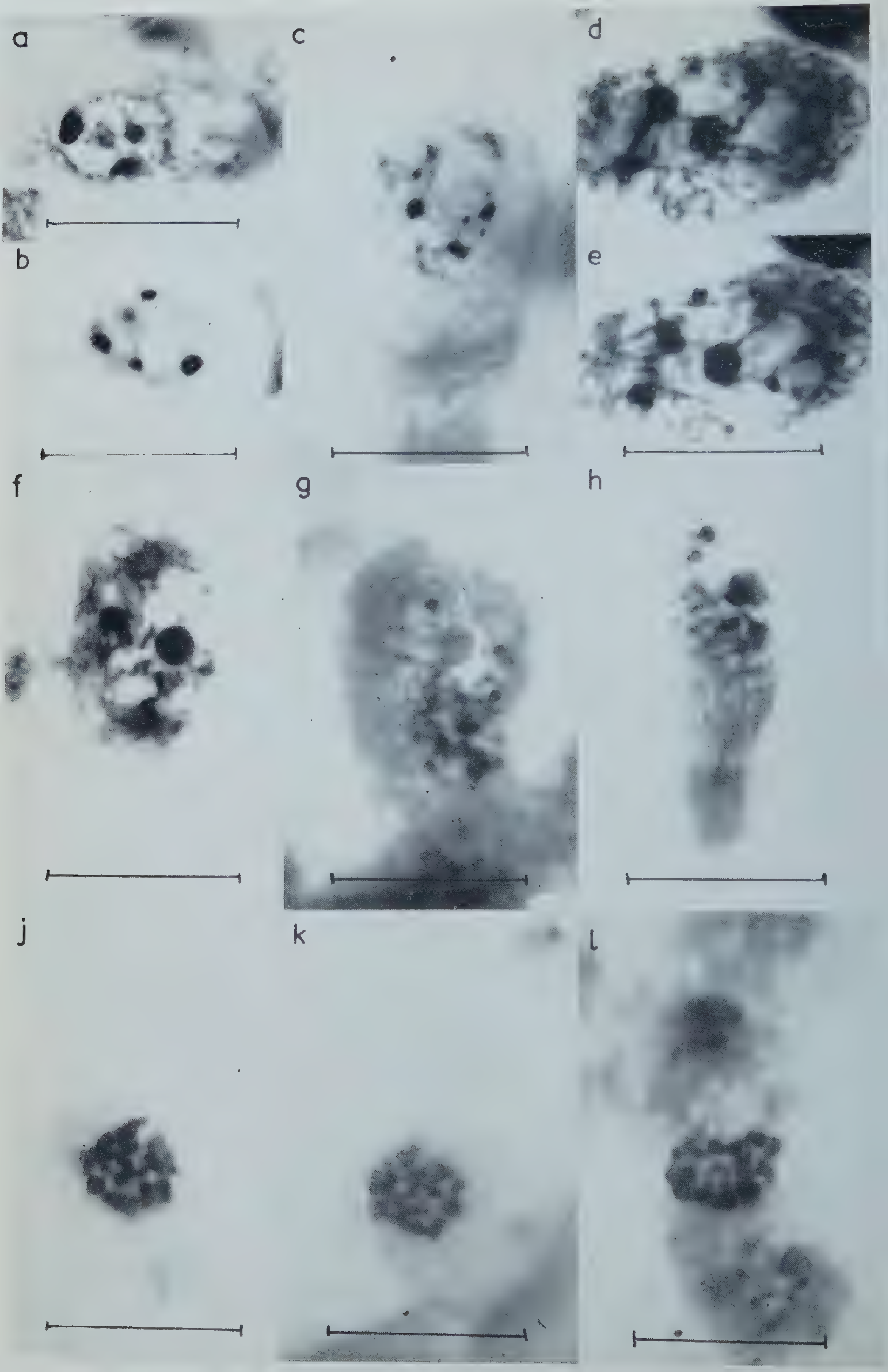
PLATE 4

Photomicrographs showing stages in the development of the oogonium; *a–c* × 2,000: *d–g* × 3,000: *h–m* × 1,500. In each case the line represents a distance of 10 μ.

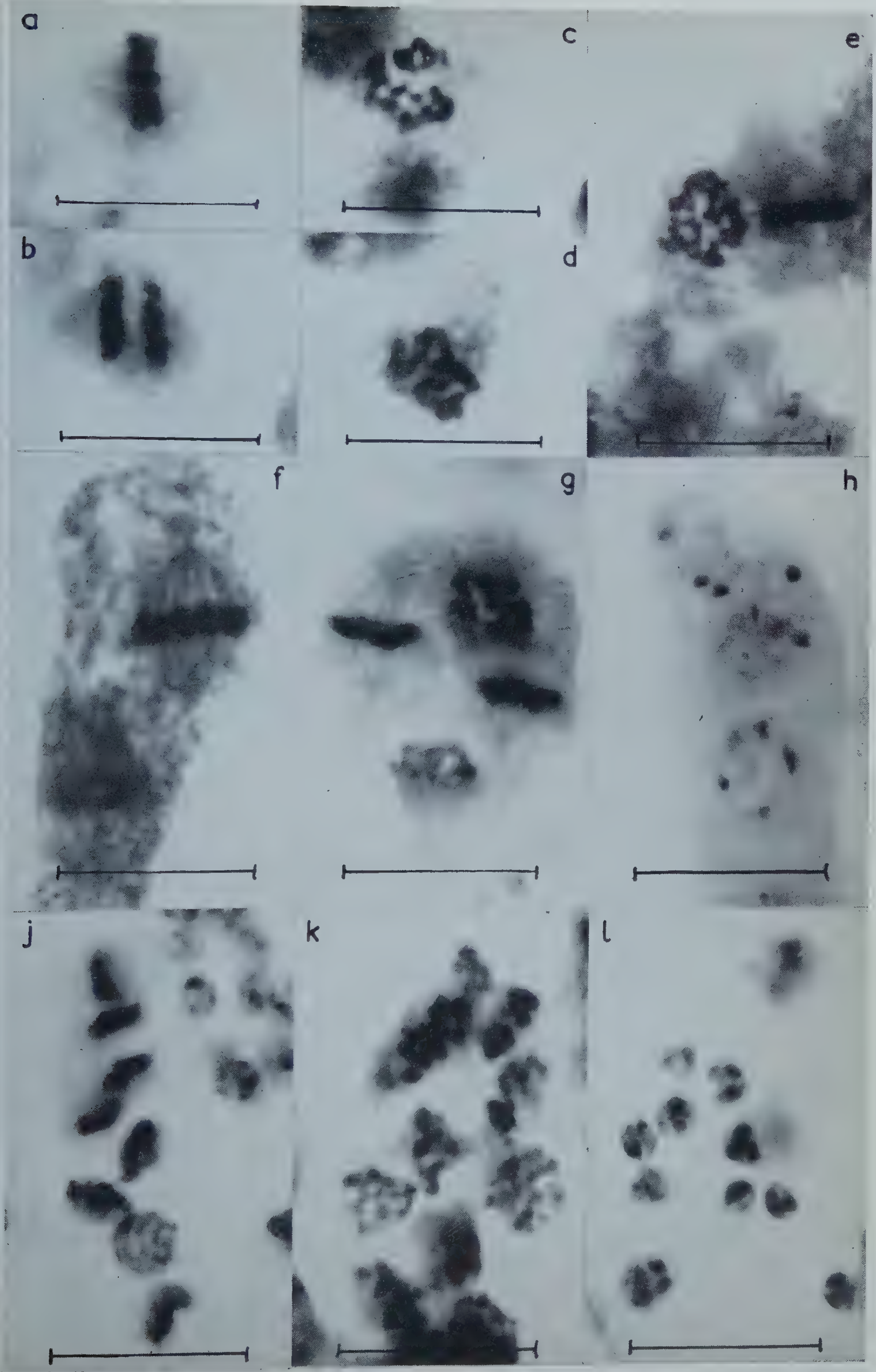
(*a*) Nucleus of an oogonium initial just before commencement of the first division, showing the very large nucleolus and 9 chromocentres. (The mark at X is a flaw on the negative.) (*b*) Early prophase in an oogonium initial showing the chromophilous spherule to the right of the nucleolus. (*c*) Early prophase in an oogonium initial showing the nucleolus and differentiation of long, slender, and spirally coiled chromosomes. (*d*) Later prophase of the first division of an oogonium initial showing the pronounced polarization of the chromosomes which are now shorter and stouter. (*e*) Polar view of a metaphase plate at the first division of an oogonium initial. Analysis of this plate gave a count of approximately 28 chromosomes. Comparison with Pl. 2*j–l* shows that this plate is of a similar diameter to that seen at the comparable division of the antheridial initial. (*f*) Side view of a metaphase plate at the second division of the oogonium initial showing the elongation of the chromosomes in the direction of the spindle, which is faintly visible. 'Spherule' not in optical plane of the photograph, cf. Pl. 3*f*. (*g*) Side view of early anaphase separation of one of the four plates at the third division; the chromophilous spherule, much diminished in size, can be seen above and to the left of the plate. (*h–m*) A series of photomicrographs, to the same scale, of nuclei at different stages of development of the oogonium. (*h*) Resting oogonium initial before the beginning of the first division, showing the nucleolus and 10 chromocentres. (*j*) Two nuclei of the quadri-nucleate stage, just at the commencement of the third division, showing the nucleolus and, in the right-hand nucleus, several small chromocentres. (*k*) Two of the nuclei at the eight-nucleate stage before the enlargement of one to form the egg nucleus; a nucleolus and several chromocentres can be seen in each nucleus. (*l*, *m*) Two planes of the egg nucleus of a mature oogonium showing the nucleolus, several chromocentres, differentiating chromosomes, and the stellate region of densely staining, finely granular cytoplasm which immediately surrounds the nucleus.

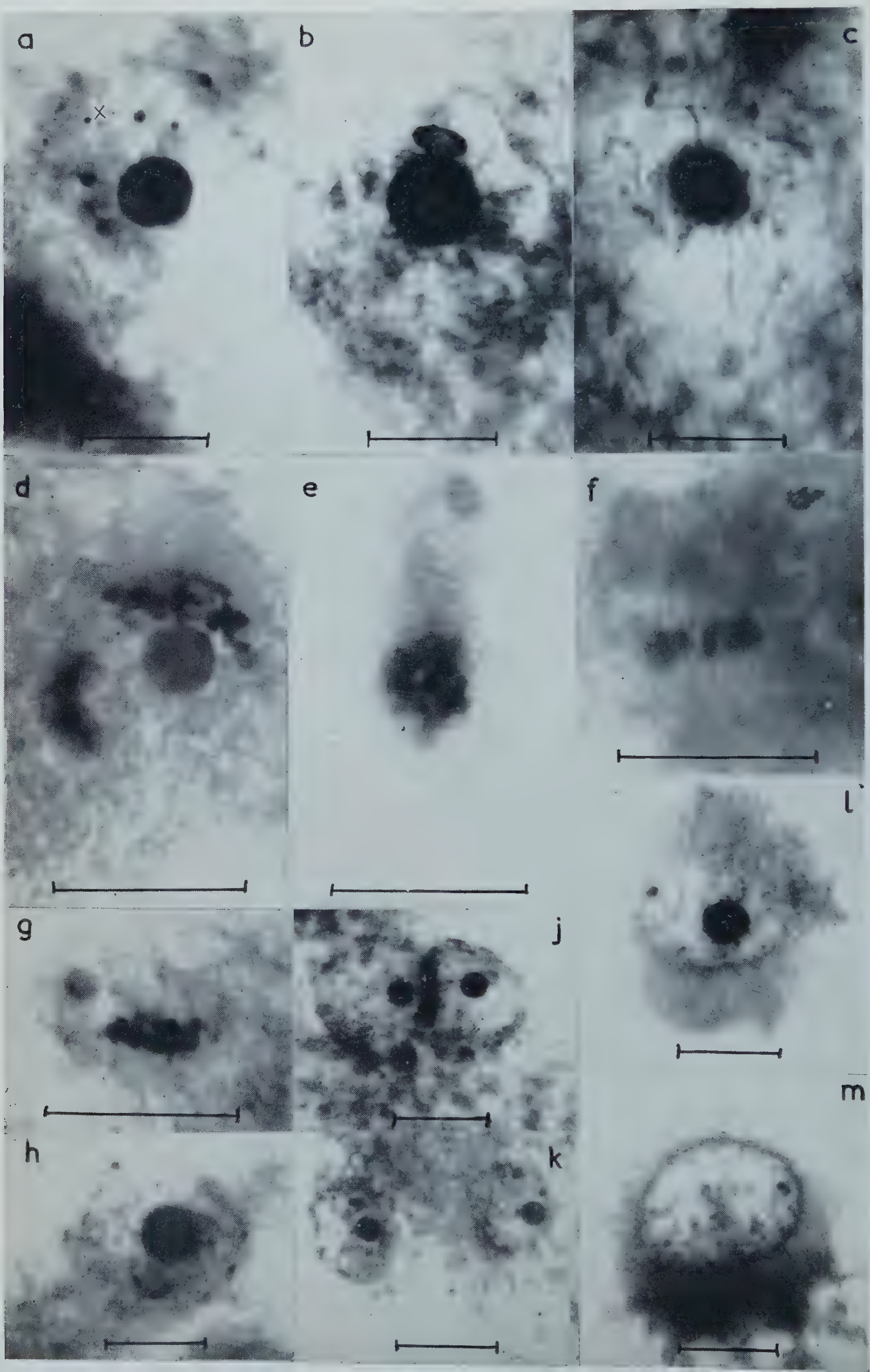


M. NAYLOR



M. NAYLOR


a
c
e
b
d
f
g
h
j
k
l


a
x
b
c
d
e
f
l
g
j
m
h
k

The Development and Behaviour of Mycelial Strands in *Merulius lacrymans* (Wulf.) Fr.

II. Hyphal Behaviour during Strand Formation

BY

GILLIAN M. BUTLER

(*Botany School, University of Cambridge*)

With seven Figures in the text

ABSTRACT

The system of hyphal branching by *Merulius lacrymans* was observed in mycelium which had grown from a wood food-base on to glass slides during incubation in sterile moist chambers. A hierarchy of branches and sub-branches arose from the region of clamp connexions, or nodes, of relatively wide main hyphae. There was evidence that the sequence of branches occurring at nodes in basipetal succession represented the time sequence of branch development at any one node. Later-formed branches at any node were smaller than earlier branches, but such earlier branches usually became smaller towards the tip as growth continued. Mycelial strands were built up by growth and branching of thigmotropically sensitive 'tendril' hyphae in association with the wide main hyphae. Tendril hyphae were characteristically narrow, thin-walled hyphae arising both as later-formed branches from the nodes of the main hyphae and as the narrowed tips of earlier branches. Although this branching behaviour could be seen amongst aerial hyphae growing over agar media, hyphae growing in contact with or within the agar behaved differently and did not form strands.

INTRODUCTION

DURING his investigations of *Merulius lacrymans* (Wulf.) Fr., Falck (1912) carried out detailed microscopical observations of the origin of strands in mycelium which had grown from small colonized pieces of wood on to glass slides in a moist chamber. He described the form of the aerial mycelium with wide main hyphae on which clamp connexions were usually visible at regular intervals of about 500μ. Branches arose in the region of these clamp connexions, or 'nodes', either from the main hypha, from the clamp arm, or from the base of an earlier branch. Falck observed the origin of characteristic 'tendril' hyphae (Rankenfäden) usually on the main hyphae from clamp connexions which had previously remained unchanged, but also from other positions including the nodes of lateral hyphae and from near the tips of short side branches. He stated that these tendril hyphae increased in length and developed clamp connexions. They could be distinguished from other branches by their initially small diameter, their irregular origin and their variable direction of growth. They were especially characterized by the way in which they grew along other hyphae with which they came into contact by their growing tips. In some instances such tendril hyphae

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

could be observed to diverge from one hypha and became applied to another. On the other hand the regular side branches in the growing region of the aerial mycelium continued to grow separately even though they were at times in close association with other hyphae. Falck observed occasional fusions of the tips of tendril hyphae with other hyphae and also H-shaped connexions between parallel hyphae but maintained that firm adherence of tendril hyphae could occur without direct fusion of parts. Although he stated that the region of formation of tendril hyphae was situated more or less far behind the mycelial margin, Falck did not make clear the position of origin of such tendrils in normally growing mycelium. Most of his observations were based on material in which growth in length at the margin was prevented without other damage to the mycelium. Under these conditions tendril hyphae formation could be observed relatively easily up to the margin of the mycelium.

From his observations Falck concluded that three main processes took part in strand formation. Firstly, the aerial mycelium tautened and individual hyphae were deflected from their position of origin and converged to form strands. This process coincided in time with the development of tendril hyphae, and Falck attributed the movements to the backward extension of the contact between tendril hyphae and those with which they were associated. He noted that these movements were difficult to follow and his account was limited to a few small places in which the process was clear. Secondly, tendril hyphae might also become applied to their parent hyphae, eventually covering the latter with a layer of tendril hyphae. Falck attributed the behaviour of tendril hyphae to their thigmotropic irritability, comparing them with the tendrils of higher plants. Thirdly, as the spaces in the strands were filled with tendril hyphae, the lumina of some of the hyphae altered in width and differentiation proceeded.

Lohwag (1938) also described in detail the development and structure of the strands of *M. lacrymans*, but his account of development largely followed that of Falck.

Observations at a macroscopic level of strand development by *M. lacrymans* (Butler, 1957) have shown that during free mycelial growth from a food-base through a non-nutrient medium, strands are initiated only in a region close behind the mycelial margin. The rates of mycelial extension and of strand development are not affected by the distance from the food-base. Accordingly microscopical observations of hyphal behaviour close to the mycelial margin were carried out under a similar system of growth. By this means it was hoped to verify the relative importance of the hyphal growth and hyphal movements reported by Falck, and also to determine the position of origin of tendril hyphae relative to the mycelial margin.

MATERIALS AND METHODS

All microscopical observations were made with the same isolate of *M. lacrymans* as had been used for observations at a macroscopic level (Butler, 1957). A few observations were made on living mycelium growing in small cells built on microscope slides (Macdonald, 1948). Although this technique was useful for observing the course of clamp connexion formation, it was not found suitable for more prolonged observations on formation of tendril hyphae. Further observations were carried out using a technique similar to that of Falck. Thin pieces of colonized wood or discs from agar cultures were placed on sterile microscope slides in sterile Petri-dishes. The slides were raised on glass rests over filter paper moistened with 3 per cent. sodium chloride solution; in order to prevent drying out, the dishes were placed in tins or polythene bags. The use of 3 per cent. sodium chloride solution did not check forward mycelial growth compared with that over water, but it did prevent undesirable condensation of water droplets on the slides.

Since observations took considerable time, a semi-permanent mounting method was required in which the minimum of disturbance to the mycelium occurred. The method selected consisted of mounting directly in Loeffler's methylene blue in glycerine (1 : 4 by vol.). In this the walls of the wide hyphae were stained pink-violet whilst the cell contents of all hyphae became blue. As it was not possible to follow the changes with time at any one point using this method, observations were made in basipetal succession from the tips of the leading hyphae of the mycelial margin. By comparing two separate sets of observations it was hoped to build up a picture of the changes with time.

SUCCESSION OF BRANCHES ARISING FROM THE MAIN HYPHAE

Characteristics of main hyphae

The mycelial margin contained a number of branching systems each consisting of a relatively wide main hypha with lateral branches which also branched to form successively smaller lateral hyphae. The same main hyphae continued to grow over distances of at least 2 cm. and only rarely did lateral hyphae of dimensions comparable with those of the main hyphae arise and act as leaders. Branching occurred almost entirely in the region of clamp connexions. The formation of a clamp connexion could be observed in the terminal cell of some of the hyphae. Where incomplete clamp arms were present the septa in both the main hyphae and the clamp arm could often be seen. Clamp connexions not associated with branches, and therefore possibly in an early stage of development, were seen more than two nodes behind the hyphal tips in only 5 out of 238 nodes examined. This suggested that the formation of clamp connexions was restricted to the terminal cells of hyphae. Septa with no clamp connexion were very rarely seen under the experimental conditions. Although the range of variation in internode length was great, the mean length of successive internodes along the hyphae changed little (Table 1). The conclusion that most growth in length occurred at the apex of each hypha was supported by direct observation of living mycelium. Thus the behaviour of nodes of the main hyphae taken in basipetal succession from the tip may be used to indicate the sequence of changes with time at any one node. Although the width of main hyphae varied from 3·5 to 7μ, changes in width along individual main hyphae were small over the first eight to ten internodes behind the tips (Table 1).

Table 1

Mean Length and Diameter of Successive Internodes of Main Hyphae of M. lacrymans

		Internodes (basipetal succession from tip)							
		1	2	3	4	5	6	7	8
A.	No. examined	36	36	36	36	35	30	27	21
	Mean length (μ)	480	720	725	660	640	600	640	645
B.	No. examined	25	25	25	25	25	0	0	0
	Mean length (μ)	475	674	613	632	609	—	—	—
	Mean diameter (μ)	5·0	5·1	5·4	5·2	5·2	—	—	—

A and B are data from two separate sets of observations.

Formation of branch hyphae

Observations were made of the branching at successive nodes along the main hyphae. Starting at one edge of each microscope slide leading hyphal tips were followed back in turn and records were made of those which did not arise from another hypha over the first eight nodes behind the hyphal tips and in which most of the clamp complexes were visible over these eight nodes. From these observations (Table 2) it could be seen that the mean number of branches per node increased at successive nodes up to the fifth node from the hyphal tip but that subsequent changes were small.

Table 2

Mean Number of Branches arising from Nodes in Basipetal Succession along Main Hyphae of M. lacrymans

		Nodes (basipetal succession from tip)							
		1	2	3	4	5	6	7	8
A.	No. nodes examined	36	36	36	36	35	35	27	21
	Mean no. branches/node	0·7	1·8	2·0	2·2	2·8	2·8	2·5	2·8
B.	No. nodes examined	24	24	24	21	20	18	0	0
	Mean no. branches/node	0·5	1·0	1·6	1·6	2·0	1·8	—	—

A and B are data from two separate sets of observations.

Apart from sprouts of uncertain fate these branches could be divided into two groups, those which were already present at the first two to three nodes behind the hyphal tips ('primary' branches), and those which first appeared at nodes farther from the tips ('secondary' branches). This difference in *time of initiation* was associated with differences in morphology and behaviour (Table 3). The structure of selected nodes is shown in Figs. 1 and 2.

TABLE 3

Comparison of the Properties of Primary and Secondary Branches of Main Hyphae of M. lacrymans

Character	Primary	Secondary
1. Time of initiation	Nodes 1 and 2 behind hyphal tip	Node 3 and later
2. Habit of growth	Nearly always independent at first	Commonly running along parent hypha (i.e. tendril habit)
3. Per cent. nodes on which one or more such branches developed during first 8 nodes from tip	100 %	45–50 %
4. No./node at eighth node	1 or 2	0–several
5. Position of origin on node	On clamp arm, sometimes also at apical end of cell, opposite clamp arm	Anywhere at apical end of cell or at base of other branches
6. Angle of insertion on parent hypha	30–60° to apex	0–180° to apex
7. Width of branch at origin	3–4 μ	*c.* 2 μ
8. Clamp connexions	Present, the first basal	Present, the first not always basal
9. Staining of wall in methylene blue	Pink-violet	Not visibly stained
10. Presence of blue-staining cell contents	Mostly at tip and apical ends of cells	Uniformly throughout
11. Rate of elongation	$<$ or $=$ parent	$<$ parent

The difference in behaviour of the primary and secondary branches was striking. Whereas almost all the primary branches were initially free, most, though not all, of the secondary branches became associated with and grew along their parent hyphae ('tendril' hyphae of Falck). Of the remaining secondary branches a few became associated with nearby wide hyphae whilst others had not come into contact with any other hyphae. The frequency of free and tendril hyphae at successive nodes (Table 4) shows that very few of the first-formed branch hyphae behaved as tendril hyphae and also that only some half of the older nodes examined had developed tendril branches.

Branches arose only in the region of clamp connexions, at the apical ends of cells. Although the protoplasmic contents which were stained in methylene blue were particularly dense at the apical ends of cells, in cells of similar age stained with basic fuchsin to show nuclei (Delamater, 1948), the nuclei in each cell were frequently in some other position along the length of the cell. Dividing nuclei, however, were not detected. Furthermore, single spore isolates of *M. lacrymans* were prepared in which clamp connexions could not be seen. Macroscopically visible strands occurred in agar cultures of some of these isolates. When this haploid mycelium was grown on to glass slides from agar inoculum discs, free and tendril branches could be seen in association with the septa, similar to those formed in the dicaryotic mycelium (Fig. 3).

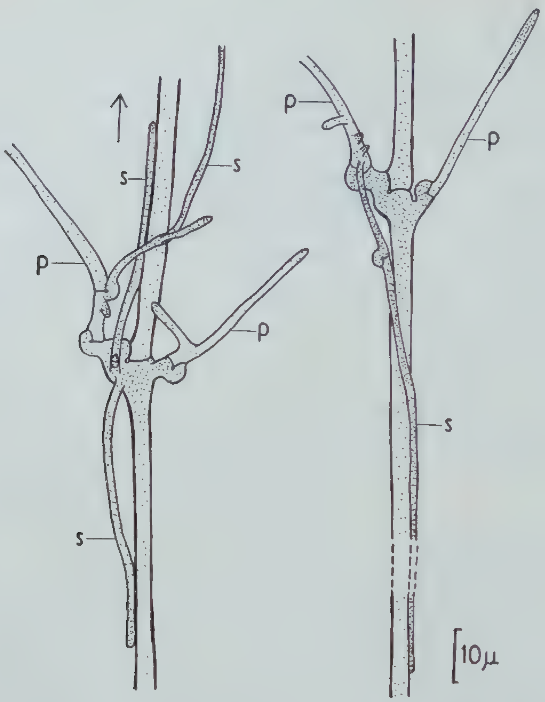


Fig. 1. Fourth (left) and fifth (right) nodes behind the tip of a main hypha of *M. lacrymans*, showing the origin of primary (*p*) and secondary (*s*) branch hyphae. Arrow indicates direction of growth of main hypha.

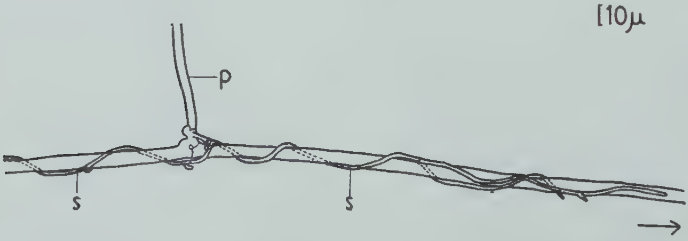


Fig. 2. Seventh node behind the tip of a main hypha of *M. lacrymans*, showing the origin and branching of a secondary (tendril) hypha.

Growth of primary branch hyphae

In considering the nature of the difference between the primary and secondary branches the fate of the primary branches was of interest. Under the conditions of this investigation only rarely did one of these branches grow and behave as a leading hypha. In some instances growth ceased altogether,

TABLE 4

Number of Free and Tendril Hyphae arising from Nodes in Basipetal Succession along Main Hyphae of M. lacrymans

(*a*) *Initially free branches*

Node*	Total no. nodes examined	% of nodes with					% of nodes with 1 or more free branches
		0	1	2	3	4	
		free branches/node					
1	36	39	58	3	0	0	61
2	36	0	72	25	3	0	100
3	36	0	55	42	3	0	100
4	36	3	64	28	5	0	97
5	35	3	46	37	14	0	97
6	35	6	54	23	14	3	94
7	27	0	59	37	4	0	100
8	21	0	48	48	5	0	100

(*b*) *Tendril branches associated with their parent hyphae*

Node*	Total no. nodes examined	% of nodes with					% of nodes with 1 or more tendril branches
		0	1	2	3	4	
		tendril branches/node					
1	36	100	0	0	0	0	0
2	36	89	11	0	0	0	11
3	36	72	28	0	0	0	28
4	36	67	25	8	0	0	33
5	35	54	29	17	0	0	46
6	35	49	29	20	3	0	51
7	27	56	26	19	0	0	45
8	21	52	24	19	0	5	48

* Nodes numbered in basipetal succession from the hyphal tip.

the tips losing their contents, and a few examples were seen in which such hyphae behaved as tendrils from their origin (Fig. 4).

Rejecting those primary branch hyphae whose tips contained no stained protoplasm, more detailed observations were made of the behaviour of the most advanced primary branch at each node. Since the number of clamp connexions on such branches arising from successive nodes of the parent hyphae followed the number on the parent hypha, it was concluded that cell division was proceeding at a similar rate in the branch and the parent hypha (Table 5). However, a comparison of the dimensions of the parent and branch hyphae arising from the fifth node of the parent hyphae (Table 6) showed that such primary branches were consistently smaller than their parent hyphae. Not only were the branches narrower at their point of origin than their parent hyphae (in 24–25 cases) but, in contrast with the main hyphae, they very frequently (in 21–25 cases) decreased in width towards the tip. It is unlikely that this tapering could be ascribed to a change in width of internodes with age, since the diameter of the basal internodes of such primary branches changed little with increasing age, compared with the changes at the tip (Table 7). This difference in diameter was associated with a change in wall thickness, since the walls of the narrowed tips were not stained pink by methylene blue. An association of two wide hyphae was rarely maintained, but such narrow primary branches appeared potentially capable of behaving as tendril hyphae, since some were observed growing along other hyphae (Fig. 5).

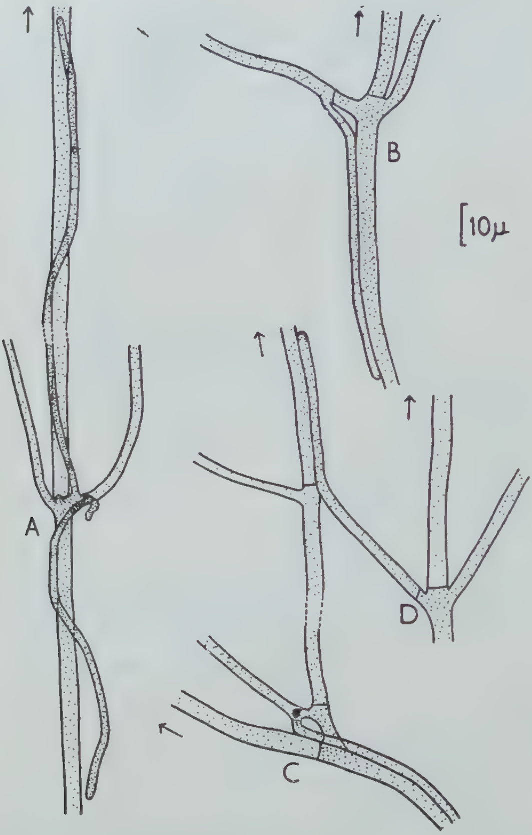


FIG. 3. Origin of free and tendril branch hyphae in single spore cultures (haploid) of *M. lacrymans*. A, third node; B, fourth node; C, fifth node; D, fourth node from the tip of a main hypha.

The reduced length of the branch hyphae (Table 6) was too great to be accounted for by the time of initiation of the branch (at the latest by the second node of the parent hypha, see Table 4). Further analysis indicated that at least two factors were involved in this size difference. Firstly a comparison of internode lengths on the primary branches showed that internode length consistently increased as the distance from the parent hypha increased. This depressing effect of proximity to the parent hypha was also reflected in a reduction of the branching at the basal (i.e. older) nodes of such primary branches (Table 8). Secondly, considering the basal three internodes of such

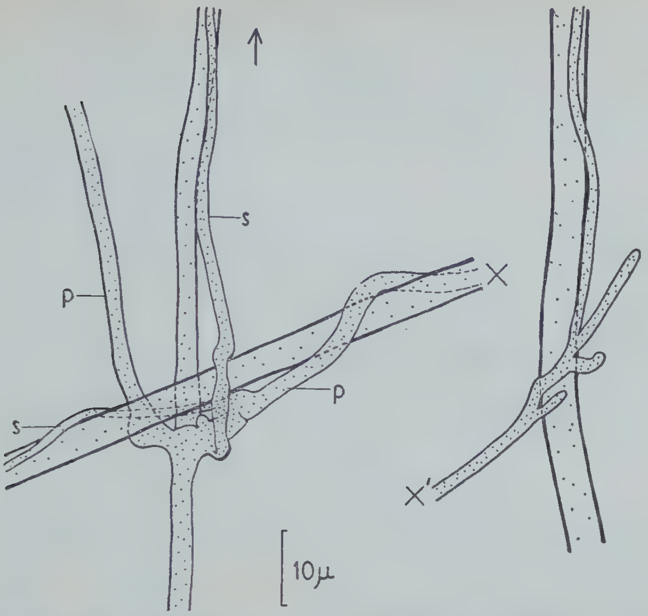


FIG. 4. Branching at the eighth node from the tip of a main hypha of *M. lacrymans*. *X* and *X′* parts of the same primary branch hypha which is behaving as a tendril first along one main hypha and then along another.

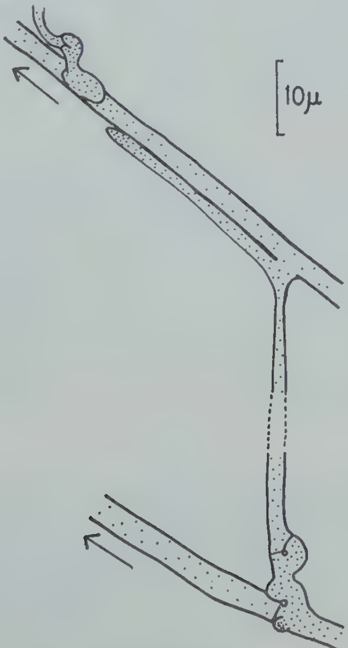


FIG. 5. The tip of a primary branch hypha behaving as a tendril on another main hypha. A hyphal fusion can be seen at the basal point of contact of the tendril hypha. The primary branch is formed on the fourth node from the tip of its parent hypha.

TABLE 5

Mean Number of Clamp Connexions on the most advanced Branch arising from Nodes in Basipetal Succession along Main Hyphae of M. lacrymans

		Nodes of main hyphae (basipetal succession from tip)							
		1	2	3	4	5	6	7	8
A.	No. examined	22	36	34	32	26	24	14	13
	Mean no. clamp connexions on branch hyphae	0·7	1·4	2·6	3·2	4·7	4·9	6·3	6·7
B.	No. examined	20	30	29	20	21	0	0	0
	Mean no. clamp connexions on branch hyphae	0·6	1·8	2·9	4·2	5·2	—	—	—

A and B are data from two separate sets of observations.

TABLE 6

Dimensions of Main Hyphae and the most advanced Branch arising from the fifth Node of these Main Hyphae of M. lacrymans

	Main*	Branch
No. observed	25	25
Mean no. clamp connexions	5·00	5·04
Mean total length (μ)	3,005	1,260
Mean internode length (μ) excluding apical cell	630	250
Mean diameter tip (μ)	5·0	2·9
Mean diameter base (μ)	5·2	4·0

* Data are for that part of the main hyphae apical to the position of origin of the branch.

TABLE 7

Diameter of the most advanced Branch Hypha arising from Nodes in Basipetal Succession along Main Hyphae of M. lacrymans

	Nodes of main hyphae (basipetal succession from tip)				
	1	2	3	4	5
No. examined	20	30	29	20	21
Mean diameter tip (μ)	4·4	3·9	4·0	3·8	3·5
Mean diameter base (μ)	4·5	4·9	4·9	4·8	4·8
% hyphae narrower at tip than base	15	80	79	81	79

TABLE 8

Properties of Successive Nodes along the most advanced Branch arising from the fifth Node of Main Hyphae of M. lacrymans

	Nodes and internodes (acropetal succession from origin of branch hyphae)				
	1	2	3	4	5
No. internodes observed	25	24	21	14	0
Mean internode length (μ)	144	233	306	332	—
No. nodes observed	25	25	24	20	15
% with 1 or more branches	48	48	67	90	60
Mean length (μ) of longest branch	tendrils	170	210	215	120

primary branches, there was an association of mean hyphal diameter with mean length of the three internodes together (Table 9).

TABLE 9

Mean lengths of Main and Branch Hyphae of Different Diameters in M. lacrymans

(*a*) *Main hyphae*

	Mean diameter (μ)						
	3·5	4·1	4·7	5·3	5·9	6·5	7·0
No. hyphae	1	3	6	4	8	2	1
Mean length (μ) of 4 internodes basal to apical cell	1,720	1,530	2,560	2,400	2,820	3,120	3,290

(*b*) *Branch hyphae arising from fifth node of main hyphae*

	Mean diameter (μ)					
	2·3	2·8	3·5	4·1	4·7	5·3
No. hyphae	1	8	3	4	2	2
Mean length (μ) of 3 basal internodes .	305	570	486	746	1,260	950

This correlation of diameter with internode length was also demonstrated where the four internodes basal to the first clamp connexion on the main hyphae were considered.

In this account of branch development the possible effect of interaction between branches and between successive nodes was not considered. The diameter at origin of successively later formed branches at any one node diminished, and the second free branch rarely grew as well as the first. Further branching of such lateral hyphae usually consisted of only one or two initially free branches and elaborate clamp complexes were not built up. Where growth of the first branch of a main hypha had ceased, quite often another progressive and fairly wide free hypha was present at the same node. An idealized diagram of the branching system in *M. lacrymans* is shown in Fig. 6.

Tendril habit

Tendril hyphae are characteristically narrow, thin-walled hyphae which arise not only from clamp connexions of the main hyphae relatively late in development, but also as the tips of narrow, originally free side branches. An analysis of the mode of origin of all the tendril hyphae which occurred on thirty-six main hyphae is shown in Table 10. This analysis indicated that although the later branches of the same hyphae formed most of the tendrils close to the hyphal tips, increasing numbers of tendrils formed from the tips of other narrow hyphae were present on older internodes.

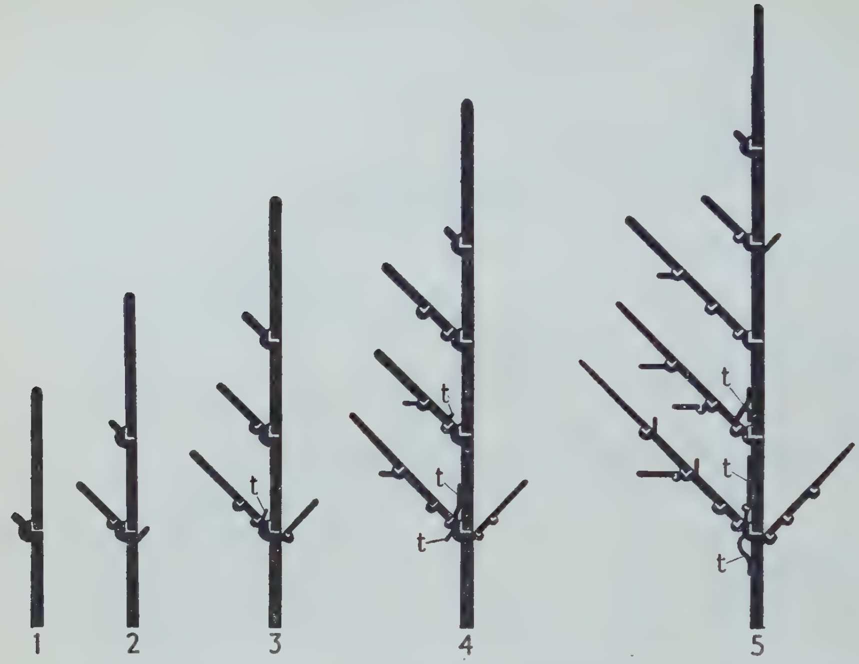


FIG. 6. Diagram showing successive stages in development of the hyphal branching system of *M. lacrymans*. *t*, tendril hypha.

TABLE 10

Origin of Tendril Hyphae occurring on Internodes in Basipetal Succession along Main Hyphae of M. lacrymans

			No. tendril hyphae				
			Arising from same hypha		Arising from different hypha		
Internode	Total no. internodes examined	% internodes with 1 or more tendril hyphae	1ry branches	2ry branches	Transferred from adjacent main hyphae	Tips of narrow hyphae	Total no.
1	36	8	0	3	0	0	3
2	36	22	0	8	0	1	9
3	36	33	0	12	0	2	14
4	35	49	3	15	2	4	24
5	35	60	2	15	4	8	29
6	30	67	1	20	2	9	32
7	27	78	1	16	2	11	30

Under the conditions of this investigation tendril hyphae were very rarely observed to arise from unchanged clamp connexions, as had been described by Falck (1912). However, as was suggested by Falck, the association of tendril hyphae with other hyphae could most readily be ascribed to a thigmotropic sensitivity of the tips of the tendril hyphae. Tendril hyphae grew both towards the tip and towards the base of other hyphae, sometimes twining spirally. Fusions between hyphae were infrequent in the early stages of growth (Fig. 5) and the associated hyphae were usually in contact along their length. Hyphae could occasionally be seen in which the tip had split into two or more narrower hyphae where it was in contact with another hypha (Fig. 4). There was no evidence of attraction at a distance since where contact between two hyphae was broken, growth often continued independently. Moreover, not all secondary branches or other narrow branches became associated with other hyphae.

DEVELOPMENT OF STRANDS

Strand initiation

A search was made for the tautening, deflexion, and convergence movements described by Falck but although hyphae apparently under tension were occasionally observed, there was no evidence that these played an important part in stranding. On the other hand, it was noticeable that tendril hyphae usually became associated with the wide hyphae which were further distinguished by walls stained pink-violet in methylene blue. The frequency of such tendril hyphae increased along the older parts of the main hyphae; furthermore, each tendril hypha continued to grow and form branches at nodes (Fig. 2). Such branches usually, but not always, behaved as tendril hyphae. Linear systems of strands were built up by the continued growth and accumulation of tendril hyphae.

Observation of the thicker strands, which could be seen some 5 cm. behind the mycelial margin on slides, showed that these strands almost always consisted of a single relatively wide central hypha surrounded by an irregular covering of narrow hyphae with deeply staining contents (Fig. 7). The covering of tendril hyphae could be seen in some instances to split up and follow both the main hypha and the primary branch at a node (Fig. 7). Occasionally the tendril hyphae were observed to pass from one main hypha to another, the course of which crossed that of the first. Out of 142 thin strands which were examined, only six were found in which a wide central hypha could not be seen. Of these six strands, two were formed by tendril hyphae passing along a narrower side branch of a main hypha covered with tendrils.

Most of the main hyphae of *M. lacrymans* gave rise to tendril hyphae, at least at some of the nodes, and could be regarded as strand initials. From the few data collected there was no evidence of a division into those which formed tendril hyphae at all nodes and those which did not form tendril hyphae (Table 11).

TABLE 11

Number of Main Hyphae of M. lacrymans *on which Tendril Hyphae were present at Nodes 5, 6, and 7 (numbered in basipetal succession from the tip)*

Tendril hyphae arising at all 3 nodes .	3
” ” ” ” $\frac{2}{3}$ ” .	9
” ” ” ” $\frac{1}{3}$ ” .	9
” ” ” ” 0 ” .	7

Nevertheless, only a small proportion of these main hyphae developed a thick covering of tendril hyphae and became strands. It was therefore necessary to postulate some other mechanism whereby preference of some strand initials for progressive development had occurred. It seems likely that strand preference was due to a chance initial advantage in food-supply from the food-base which would be magnified as growth continued. Strand preference has also been observed amongst strands 15 μ or more in diameter (Butler, 1957).

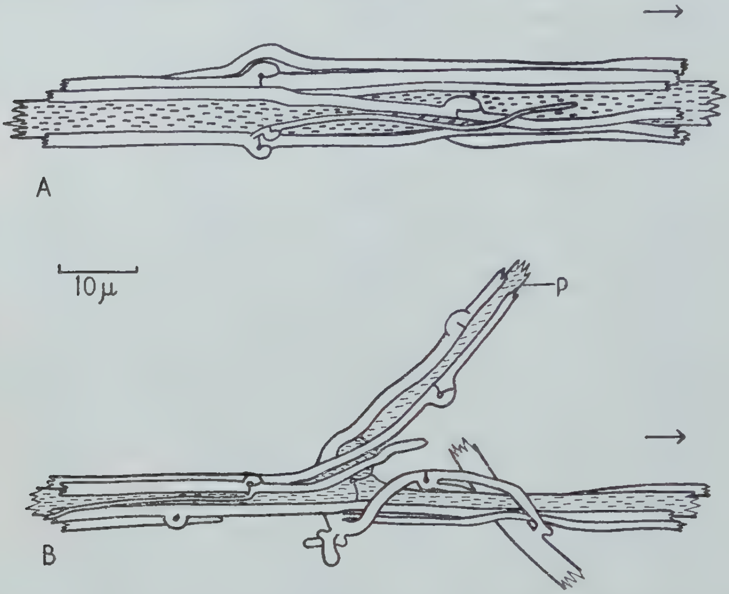


Fig. 7. Surface view of thin strands of *M. lacrymans* (main hyphae are stippled). A, wide main hypha with a covering of narrower tendril hyphae; B, tendril hyphae growing along a wide main hypha and its free primary branch (*p*).

Strand differentiation

The differentiation of strand tissue into three principal types of hyphae, vessel hyphae (Gefässhyphen), fibrous hyphae (Faserhyphen), and structural hyphae (bildenden Fäden) has been described in great detail by Falck (1912) and Lohwag (1938). Both workers reported that the other hyphal types were differentiated from structural hyphae. On the basis of their similarity in diameter (*c.* 2 μ), wall thickness, and branching it seems likely that the structural hyphae were formed from the growing and branching tendril hyphae of the developing strands. No attempt was made to follow this further strand differentiation, but it was of interest to find out how far differentiation occurred under the cultural conditions used. Pieces of thicker strands were removed from some of the tubes of the porous pot experiments (Butler, 1957) after growth for 14 and 16 weeks and were teased apart in 3 per cent. potassium hydroxide solution. These strands were covered with a layer of loosely arranged narrow, branching hyphae (structural hyphae), but differentiated hyphae could be seen within. Thick-walled fibrous hyphae were frequent to abundant and vessel hyphae 10–20 μ in diameter, some with the characteristic 'beam' and 'ring' wall thickenings described by Falck, were present. Similar hyphal differentiation could be seen in strand fragments from plate cultures of a similar age on nutrient agar.

HYPHAL DEVELOPMENT ON AGAR

M. lacrymans formed mycelial strands over agar both during growth from a food-base on to distilled water agar and in growth over nutrient agar (e.g. Czapek–Dox agar). It was observed that the form of the mycelium when growing from a food-base on to distilled water agar (Difco Bacto-agar) was different from that on glass slides. Those hyphae, which were in contact with the agar surface or were growing submerged within the agar, were usually narrower and followed a more winding course. The distance between clamp connexions was shorter. The narrow lateral hyphae branched repeatedly, not always at clamp connexions, and the angle of insertion of branches was frequently high, 60–90°. A similar hyphal behaviour was observed amongst hyphae growing from a food-base over the surface of cellulose film placed on the water agar. Neither tendril hyphae nor strands were seen in such substrate mycelium whereas hyphae not in contact with the medium ('aerial' hyphae) formed tendril branches and were similar to the hyphae which grew over glass. There was an interchange of hyphae between the substrate and aerial mycelium, so that the difference was a response to environmental conditions.

SUMMARY AND CONCLUSIONS

The behaviour of individual hyphae of *M. lacrymans* was observed during growth from a wood food-base over glass slides. It was clear that *M. lacrymans* is a translocating fungus, as defined by Schütte (1956), and that all nutrients for the growth of the hyphal apices were obtained from the food-base through the older parts of the mycelium. The same main hyphae continued to grow in the leading margin with the same diameter and the same mean internode length over distances of 2 cm. or more. Cell division, with formation of clamp connexions, was restricted to the apical cells and branch hyphae were present almost always in association with such clamps. A sequence of branch development could be observed at nodes taken in basipetal succession from the tips of the main hyphae. It is probable that this spatial sequence represents a sequence in time at any one node.

A hierarchy of branches arose from each main hypha. Not only did these wide hyphae give rise to lateral branches which subsequently branched to form further orders of branches, but also successive branches were formed over a considerable period of time at each node of the main hyphae and their wider branches. Such lower orders of branches in both space and time were successively smaller at origin until a minimum diameter of *c.* 2 μ was reached. This difference in size included changes in hyphal diameter, internode length, wall-staining properties, and ability to behave as tendrils. Changes in some of these properties occurred with age, but were negligible over these early stages of growth. Moreover, the diameter of many of the originally wider branches diminished as growth proceeded so that there was a tapering of such hyphae from their origin. It was also observed that both internode length and sub-branch development were reduced on lateral hyphae close to their origin on the parent hyphae, compared with more distal parts. Although all branches were potentially capable of unlimited growth, only a few attained dimensions comparable with their parent main hyphae under the conditions of growth. Cessation of growth of main hyphae was usually associated with the occurrence of larger branch hyphae.

The ability of a node of an intact main hypha to form lateral branches was limited by the maturity of the node and the diameter of the hypha. New branches were initiated from nodes of main hyphae up to five nodes from the hyphal tips. Moreover, although narrower hyphae very rarely formed more than two branches at any one node, up to seven branch initials were observed to arise from one clamp complex of a wider hypha (*c.* 6μ in diameter).

The use of observations of successive nodes as a relative time scale precluded discrimination between differences in the rate of clamp formation and differences in the rate of apical elongation of individual hyphae. Nevertheless it was clear that some form of apical dominance maintained the branch hierarchy. Although there is no direct evidence of the mechanism of such dominance a nutritional mechanism could explain most of the observations. The restriction of hyphal branching in aerial mycelium to the region of clamp connexions has been noted in many homobasidiomycetes including *Mycena sanguinolenta* (Forsyth, 1950) and *Polyporus cinnabarinus* (Routien, 1948). In the latter the formation of several branches of different ages in association with a single node of a main hypha was described. In *Merulius lacrymans* the abundance of protoplasmic contents densely stained by methylene blue was the only morphological feature distinguishing the branch-forming apical ends of cells from other parts of the cells. A similar system of main hyphae with a hierarchy of branch hyphae may be observed in many fungi, e.g. *Phymatotrichum omnivorum* (Rogers and Watkins, 1938) and *Pyronema confluens*. Of the morphological features of the different hyphae recorded for *M. lacrymans* the size of the various hyphal apices is probably most fundamental. The diameter of each initial is, in its turn, related to the dimensions of the parent node and to the space available after formation of earlier branches. The shorter internode length and reduced branching of each lateral hypha of *M. lacrymans* close to its origin was similar to the observation of Henderson Smith (1924) that the rate of extension of each branch hypha of *Botrytis cinerea* increased with time from its initiation. This observation of *B. cinerea* was made during growth on nutrient agar. Since this reduction of growth in *M. lacrymans* was also reflected in branching behaviour it could not be due to the immaturity of the apex of the branch at initiation.

As had been described by Falck (1912) strands were formed largely by growth and branching of tendril hyphae which characteristically became associated with, and grew along, other hyphae with which they came into contact. Observations support the view that the response is thigmotropic. Thigmotropic sensitivity was limited to the narrower hyphae. A chance association of two wide hyphae was not maintained, but all the narrow hyphae appeared potentially capable of the response whether they arose late from the nodes of the main hyphae, or as the narrowed tips of earlier branches. Compared with wide hyphae, such narrow hyphae had thinner walls and showed greater variations in direction of growth within the same distance.

Although two narrow hyphae were sometimes seen growing in contact, under the experimental conditions on slides each strand was built up around one of the original main hyphae and not as an independent association of tendril hyphae with one another. It is unlikely that this behaviour can be attributed to a difference in ability of narrow and wide hyphae to act as a surface for growth of tendril hyphae; more probably this behaviour depends on the greater chances of contact with wide hyphae early in mycelial development and on the better nutrient supply of the wider hyphae. This nutritional explanation could further be applied to the preference of some of the strand initials for progressive development into thick strands.

A similar development of strand-forming tendril hyphae has been described not only in other wood-destroying fungi e.g. *Coniophora cerebella* (Falck, 1912) and *Paxillus panuoides* (Findlay, 1932), but also in some strand-forming mycorrhizal fungi (Melin, 1923; How, 1940). The development of strands in *Phymatotrichum omnivorum*, which was described by Rogers and Watkins (1938), is similar to that of *M. lacrymans*. In both fungi strand development is preceded by general mycelial growth and each strand is built up by growth of branch hyphae around their parent main hyphae. Moreover, this ensheathing does not proceed uniformly along all parts of the main hyphae and appears to begin in a restricted physiological age of mycelium in both fungi. However, in *P. omnivorum* each central hypha is much wider and more clearly defined and increase in thickness is limited relatively early by maturation of a dark coloured cortex.

Observations of growth on agar plates have shown that the behaviour of hyphae growing in or in contact with the substrate is different from that of aerial hyphae. Strand development is restricted to the aerial hyphae which are not in direct contact with nutrients. This difference is a response to environmental conditions. A comparable difference in behaviour of aerial and substrate mycelium occurs in many fungi. Buller (1924) noted in *Psathyrella disseminata* a well-defined distinction between ordinary vegetative mycelium, consisting of thin-walled colourless hyphae growing into the nutritive substratum and the 'ozonium', which developed at the expense of the vegetative mycelium and grew away from the nutritive substratum. The 'ozonium' consisted of thick-walled red hyphae which anastomosed to form strands. This behaviour may be compared with the restriction of development of other specialized structures, e.g. spore-bearing hyphae, to the aerial mycelium. By contrast with *Pyronema confluens* in which development of aerial mycelium, including apothecia, lags behind growth of the substrate mycelium (Robinson, 1926), growth of aerial hyphae in *M. lacrymans* is as rapid as, or more rapid than, that of substrate hyphae. The development of aerial hyphae and of strands by cultures of *M. lacrymans* on various nutrient media will be described more fully in a subsequent paper.

ACKNOWLEDGEMENTS

I am indebted to Dr. S. D. Garrett for his advice and encouragement. My thanks are also due to Girton College, Cambridge, for an Ethel Sargant Research Studentship and to the Department of Scientific and Industrial Research for a maintenance grant during the tenure of which this work was done.

LITERATURE CITED

BULLER, A. H. R., 1924: Researches on Fungi. 3. Longmans, Green & Co., London.

BUTLER, G. M., 1957: The Development and Behaviour of Mycelial Strands in *Merulius lacrymans* (Wulf.) Fr. 1. Strand Development during Growth from a Food-base through a Non-nutrient Medium. Ann. Bot., N.S., Lond., **21**, 523–37.

DELAMATER, E. D., 1948: Basic Fuchsin as a Nuclear Stain for Fungi. Mycologia, **40**, 423–9.

FALCK, R., 1912: Die Merulius Fäule des Bauholzes. Hausschwammforsch., **6**, 1–405.

FINDLAY, W. P. K., 1932: A Study of *Paxillus panuoides* Fr. and its Effects upon Wood. Ann. appl. Biol., **19**, 331–50.

FORSYTH, E. M. E., 1950: A Study of *Mycena sanguinolenta*. Trans. Brit. mycol. Soc., **33**, 24–34.

HOW, J. E., 1940: The Mycorrhizal Relations of Larch. 1. A Study of *Boletus elegans* Schum. in Pure Culture. Ann. Bot., N.S., Lond., **4**, 135–50.

LOHWAG, H., 1938: Mykologische Studien—14. Zur Anatomie des Strangmyzels von Gyrophana lacrymans (Wulf.) Pat. Ann. mycol., Berl., **36**, 401–34.

MACDONALD, J. A., 1948: An Improved Technique for the Study of Living Mycelium. Trans. Brit. mycol. Soc., **31**, 92–93.

MELIN, E., 1923: Experimentelle Untersuchungen über die Konstitution und Ökologie der Mycorrhizen von *Pinus silvestris* L. und *Picea abies* (L.) Karst. Mykol. Untersuch. u. Ber. von R. Falck., **2**, 73–335.

ROBINSON, W., 1926: The Conditions of Growth and Development of *Pyronema confluens* Tul. (*P. omphaloides* (Bull.) Fuckel). Ann. Bot., Lond., **40**, 245–72.

ROGERS, C. H., and WATKINS, G. M., 1938: Strand Formation in *Phymatotrichum omnivorum*. Amer. J. Bot., **25**, 244–6.

ROUTIEN, J. B., 1948: Hyphal Proliferation through Clamp-formation in *Polyporus cinnabarinus* Fr. Mycologia, **40**, 194–8.

SCHÜTTE, K. H., 1956: Translocation in the Fungi. New Phytol. **55**, 164–82.

SMITH, J. HENDERSON, 1924: On the Early Growth Rate of the Individual Fungus Hypha. Ibid., **23**, 65–78.

Translocation and Pileus Formation in *Polyporus brumalis*

BY

B. E. PLUNKETT

(*Birkbeck College, London*)

With Plate

ABSTRACT

The already-known promotion of pileus formation in pure cultures of *Polyporus brumalis* by heightened evaporating conditions is shown to be associated with enhanced translocation rates into the fruit-body (as investigated by dyes). This does not result in changed proportions of dry matter or potassium in the tissues at the stipe apex, but accelerated growth (dry-weight basis) occurs in this region. Reduction of concentration of the medium (and hence probably of the concentration of the translocation stream) diminishes pileus production. Light intensity has only a minor effect on dye transport. Fruit-bodies arrested in growth or killed show a negligible capacity for dye transport.

INTRODUCTION

IN an earlier paper (Plunkett, 1956) it was shown that heightened transpiration rates of the developing sporophores of *Polyporus brumalis* (Pers.) Fries resulted in hastened pileus production, increased numbers of pileate fructifications, and increased cap diameters. This suggested a hypothesis, tested in the experiments which follow, that the promotion of pileus formation with increasing transpiration is due to enhanced rates of transport up the stipe of material essential to cap development. Although the experiments had this limited purpose, they shed a little light on the problems of mechanism of transport in hymenomycete sporophores as well.

The developing fruit-body, unlike the vegetative mycelium, it not in contact with the nutrient substratum and must, therefore, depend for its structural materials and energy source upon a translocation stream ascending the stipe.

Buller (*Researches on Fungi*, v, 1933, pp. 147–50) has given a picture of transport into fructifications based on analogy with his and other workers' detailed observations of protoplasmic streaming in the vegetative mycelium. His thesis is that pressure from below, developed as a result of the formation of new protoplasm and vacuoles in the mycelium, causes an acropetal flow of protoplasm into the sporophore. For the purpose of this paper this will be called 'mycelium pressure'.

That materials other than water do flow into the fruit-body during development is proved by its increasing dry weight, as shown for *Agaricus campestris* (Bonner *et al.*, 1956), *Collybia velutipes* (Plunkett, 1951), and *Coprinus lagopus*

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

(Madelin 1956 *a* and *b*). Madelin's experimental demonstration that this may be accompanied by a temporary loss in weight of the mycelium is in harmony with Buller's conception of an emptying (vacuolating) vegetative hyphal system, but does not indicate the motive force of translocation.

A flow into the fruit-body actuated by 'mycelium pressure' probably implies an intrahyphal route of transport. In this connexion it is important to note that the septa of sporophore hyphae in hymenomycetes possess a central pore, like those of the mycelium, through which protoplasm might flow (Wahrlich 1893).

It has long been known, however, that the final rapid stages of cap and stipe expansion in *Coprinus spp.* may occur in sporophores detached from the mycelium (references in Voderberg, 1950). Such growth may be a matter of cell expansion, perhaps accompanied by redistribution of materials, but will involve no dry-weight increase. Schütte (1956) has shown that dye ascends rapidly into young sporophores of various agarics detached from their mycelia and with the stipe bases placed in watery solutions of the marker. As in the case of protoplasmic streaming towards aerial parts in certain vegetative hyphae (references in Buller, 1933 and Schütte, 1956) this was strongly correlated in rate with transpiration intensity and, like it, varied with the saturation deficit of the air. When pileus transpiration was reduced by a vaseline film a parallel and proportionately similar reduction of dye transport ensued. Up to this point the results suggest that radiant energy, acting through evaporation, actuates the process. However, transpiration (weight loss) and ascent of dye were claimed to suffer no great reduction in a saturated atmosphere and also to decrease in a parallel fashion as the fruit-body became mature. Here is evidence that transpiration and the strongly correlated translocation are dependent upon a vital process which, although sensitive to evaporation, is mainly actuated otherwise than by evaporational suction.

Upward transport by evaporational suction need not necessarily be envisaged as following an intrahyphal route. The tightly packed hyphae of the fruit-body could act as a wick, ascent occurring under the influence of purely physical forces. Nevertheless Schütte has established that dye ascent in mature agaric (but not polypore) fructifications is localized to a zone adjoining the stipe cavity and at the junction of the lamellae and pileus flesh. This and his data suggesting an important vital component of transpiration may perhaps be construed as evidence for an intrahyphal pathway of translocation. At the present state of knowledge interhyphal transport cannot be ruled out and, again, it might co-exist and co-operate with transport inside the hyphae.

Thus on present hypotheses transport is actuated by mycelium pressure or evaporational suction or an unknown vital mechanism which is not mycelium pressure. Such views are not necessarily mutually exclusive and all or some of them could perhaps be valid at the same or different times in the same sporophore.

The above discussion of transport mechanisms is based almost wholly on evidence from Agaricales, which usually grow, after an early stage, by a process of inflation of pre-formed cells. *P. brumalis* grows mainly by formation of new hyphae in the distal part of the sporophore. It is not clear how this may affect transport problems, but it should, perhaps, not be disregarded in comparing results from agarics and polypores.

It may be re-emphasized that the experiments which follow were designed to find a rationale for the promotion of pileus formation in *P. brumalis* by heightened evaporating conditions.

METHODS

Full details of the technique used in producing fruit-bodies of *P. brumalis* in pure culture may be found in an earlier paper (Plunkett, 1956).

The cultures were grown on a 4 per cent. malt extract solution in small vessels contained in a glass chamber, through which sterile air of controlled relative humidity (R.H.) could be passed. Losses of water by evaporation from the culture vessels were replaced by an automatic device. Cultures were continuously illuminated by fluorescent tube lamps and maintained at 20° C.

For most of the present experiments two contrasting conditions of evaporation were used. 'Rapid' transpiration was obtained by passing dry air (0 per cent. R.H.) into the culture chamber at 200 ml./min. Under such conditions transpiration per unit surface of the fungus falls as the surface increases during fruit-body development. In previous experiments an estimated initial value of *c.* 6·5 at primordium production fell to *c.* 1·5 mg./cm.^{2}/hr. at maturity. 'Slow' transpiration was obtained by passing air of approximately 96 per cent. R.H. into the chamber at 5 ml./min., resulting in a range of water loss of *c.* 0·001 to *c.* 0·0005 mg./cm.^{2}/hr. (Plunkett, loc. cit.).

In developing *P. brumalis* low light intensities tend to prevent pileus production even with rapid evaporating conditions and high light intensities to partly counteract the pileus inhibiting effect of low transpiration. Thus in some of the present experiments light intensity was adjusted to an intermediate value of about 60 f.c. to allow full expression of the transpiration effect on cap production. When, however, transpiration rate was not the variable investigated, various other combinations of evaporation rate and light intensity were applied with a view to producing conditions in which the extent of pileus development would be sensitive to external factors.

Other details are given in connexion with individual experiments. The mode of presentation and analysis of results is as in the previous communication (Plunkett, loc. cit.).

1. INDIRECT EVIDENCE OF THE ASSOCIATION OF CAP DEVELOPMENT AND TRANSPORT RATE

Effect of Medium Concentration

If evaporating conditions influence cap development through the rate at which materials are carried to the stipe apex, it might be expected that a reduction of the concentration of the medium bathing the mycelium would have much the same effect on cap development as lowered evaporation. The assumption is here involved that concentration of materials in the translocation stream is related to medium concentration.

In a first experiment, groups of cultures in separate glass chambers were grown at fairly high light intensity (150 f.c.) under 'slow' evaporation conditions (see Methods, and Plunkett, 1956). In previous work (loc. cit.) it has been shown that such conditions tend to result in the formation of rather small pilei, a state sensitive to small changes of environment and hence suitable for the investigation of factors which may affect capping. Three groups of cultures were grown on 0·26, 0·86, and 4·3 per cent. initial concentrations of malt extract.

No fruiting occurred on the most dilute medium, but at the other concentrations some sporophores arose which showed certain morphological differences in relation to medium concentration (Table 1).

TABLE 1

The Effect of Initial Concentration of Malt Extract in the Culture Medium upon the Dimensions (mm.) of Pileate Fruit-bodies developing at 150 f.c. Light Intensity and Slow Evaporation Rates

Concentration of malt extract %					
4·3			0·86		
Stipe length	Cap diam.	Stipe diam.	Stipe length	Cap diam.	Stipe diam.
40·3±17·1* (6)†	12·3±0·67	2·66±0·11	36·0±7·6 (3)	4·0±0·58	1·33±0·17

* Mean ± standard error of the mean.

† In this and subsequent tables the size of the sample is indicated in parentheses.

The three pileate fruit-bodies produced on the weaker medium had significantly smaller pilei and narrower stipes than those on the richer medium ($P = < 0{\cdot}01$), although stipes were of comparable length. It was deemed a disadvantage of this experiment that exhaustion of the weaker medium by the time of pileus formation rather than slow rate of supply of nutrients might account for the results. The next experiment partly reduces this possibility by delaying the reduction of medium concentration from the beginning of the experiment to the stage when short stipes (10–20 mm.) were formed, but before the appearance of caps.

Groups of cultures were inoculated upon 5 per cent. malt extract and grown under conditions of 'slow' evaporation (Methods) at a light intensity of 60 f.c., that is, under conditions which discourage pileus production. At 12 days, when some short stipes had appeared, about four-fifths of the medium was withdrawn from one set of cultures and replaced by sterile water, using a sterile pipette. This and an undiluted set of cultures were then placed under conditions more favourable to cap formation (light intensity 160 f.c., dry air entering the culture-chambers at 40 ml./min.). A further group of untreated cultures was left as a control under the original low light and evaporation conditions.

Again it was seen (Table 2) that a low medium concentration, even when applied to previously richly nourished mycelia and primordia, significantly reduced the final diameters of pilei as compared with those in parallel but undiluted cultures ($P = < 0\cdot01$). Also, despite more favourable light and evaporating conditions, the diluted cultures did not differ in this respect from the controls at low light intensity and with low evaporation rates ($P = 0\cdot6$–$0\cdot5$).

TABLE 2

The Effect of Reducing Medium Concentration during the Development of Fruit-bodies upon their Final Dimensions (mm.) as compared with Undiluted Controls at the same and at lower levels of Light Intensity and Evaporation

Low light and evaporation throughout*			High light and moderate evaporation level during cap development*					
Undiluted medium			Diluted medium			Undiluted medium		
length	Cap diam.	Stipe diam.	length	Cap diam.	Stipe diam.	length	Cap diam.	Stipe diam.
42·7 ± 7·9 (3)	9·7 ± 1·2	2·33 ± 0·17	37·4 ± 3·7 (8)	11·4 ± 1·4	1·75 ± 0·16	42·6 ± 6·4 (5)	23·8 ± 1·7	3·3 ± 0·44

* See text.

Some fruit-bodies in the undiluted cultures which had been transferred to conditions favourable to capping showed a remarkable increase in thickness, following the change-over, in the newly formed parts of the stipe. It did not occur in two fructifications which produced caps within a day of the transfer, thus virtually forming no new stipe tissue (Plate No. 6). If maximum stipe diameters are compared with those of the diluted cultures a significant difference emerges ($P = < 0\cdot01$), but this is not obtained upon comparison with the moist, ill-illuminated control ($P = 0\cdot2$–$0\cdot1$) due no doubt to the small size of one of the samples and the variability introduced by only some of the stipes showing enhanced thickness following changes of light and evaporation. Sensitivity of stipe diameter to external conditions has been noted previously (Plunkett, 1956). As in the last experiment the conditions applied resulted in no meaningful differences in stipe length.

In both experiments, but more especially in the second, it is clear that reduction of medium concentration adversely affects cap development in a fashion parallel to low transpiration rates. This is in harmony with the thesis that pileus development is dependent on the quantity of materials arriving at the stipe apex in a given time, as this would be affected both by the concentration of a transport stream and its rate of flow.

The Dry Matter and Potassium Content of Fruit-body Apices

If materials travelled more rapidly to the apices of fruit-bodies growing in dry than in wet air and were not instantly utilized, analysis might reveal quantitative chemical differences between the two kinds of apex. This possibility was investigated in two experiments.

Firstly, determinations were made of the proportion of dry matter in apices grown under the contrasted conditions of 'rapid' and 'slow' evaporation (see Methods). Light intensity was maintained at 60 f.c. Fruit-bodies between 8 and 13 mm. in length were removed from the cultures. Early stages of cap formation were often evident in those from the higher evaporation sample. The terminal 3 mm. of the fruit-body was cut off with a sharp razor and weighed, then dried for 24 hours at 80° C. and re-weighed. Dry weights as a percentage of fresh weights were calculated (Table 3). No suggestion of a difference between the two samples was obtained.

TABLE 3

The Mean percentage Dry-Matter Content of young Fruit-body Apices from Conditions of Slow and Rapid Evaporation

Rapid evaporation*	Slow evaporation*
23·4±1·8 (7)	21·4±0·6 (20)

Comparison of means $P = 0{\cdot}2\text{–}0{\cdot}1$

* See Methods.

Secondly, a specific element, potassium, chosen for its ease of assay in very small quantities, was determined in two groups of apices similarly contrasted in transpiration conditions. Dry weight was determined and then the apex was ground up in 12 ml. of distilled water at 80° C. Upon cooling K was estimated using an 'EEL' flame photometer. The samples were closely similar and obviously not to be regarded as differing statistically (Table 4).

TABLE 4

Mean K Content (as percentage dry matter) of Young Fruit-body Apices Grown under Rapid and Slow Evaporation Conditions

Rapid evaporation*	Slow evaporation*
4·5±0·19 (11)	4·3±0·24 (13)

Comparison of means $P = 0{\cdot}6\text{–}0{\cdot}5$

* See Methods.

These results lend no support to the suggestion that enhanced evaporation conditions result in a greater concentration of materials at the apex. This would accord with the hypothesis of more rapid ascent under such conditions only if a speedier conversion of materials into fungus tissue occurs at the apex in drier air. This led to the following attempt to measure growth-rate (basis of dry weight) at the two types of apex.

The Growth-rate of Fruit-body Apices

In two groups of experimental chambers illuminated at 60 f.c. and having 'rapid' and 'slow' evaporation conditions respectively, epileate fruit-bodies of approximately 10 mm. in length were marked at the extreme apex using indian ink on a soft camel-hair brush. Four days (96 hours) later the tips had grown beyond the mark. The ink, too, had spread beyond its original limits owing to cell enlargement. New growth, lying above the uppermost visible part of the mark, was then severed from the rest by a cut at right angles to the long axis of the stipe. It is assumed that over a number of individual operations any error due to estimating the upper limits of the ink mark would be randomly distributed and introduce no bias. Regardless of conditions some apices grew little compared with parallel fruits or failed to do so at all. Such abortion is a common feature of cultures of *P. brumalis*. Only cases of complete failure to grow after marking were discarded. Later abortion was, obviously, indistinguishable from very slow growth and could only be catered for by taking a fairly large sample.

TABLE 5

Dry Matter Increment (*mg.*) *in* 96 *hours at the Apices of Young Fruit-bodies forming in Rapid and Slow Evaporation Conditions*

Rapid evaporation*	Slow evaporation*
4·23±0·9 (16)	1·02±0·24 (21)

Comparison of means $P = < 0{\cdot}01$.

* See Method.

Despite these possible difficulties a much increased growth-rate was shown to be associated with the more rapid evaporation conditions (Table 5). During the period between marking and harvesting all but three of the rapidly transpiring fruit-bodies produced pileus rudiments. However, these three specimens showed good growth with a mean increment of 2·6 mg. which exceeded that of the low-evaporation sample ($P = < 0{\cdot}05$). A single pileate fruit-body produced at low evaporation rates showed a high growth-rate, but being unique did not lend itself to comparisons. Clearly, apical growth-rate differences of a high order exist between sporophores in the two experimental conditions. How far this is the cause of, is merely parallel to, or proceeds from, cap formation is not certain. It is good evidence of increased transport rates under more rapid evaporation conditions.

2. DIRECT EVIDENCE OF THE ASSOCIATION OF CAP DEVELOPMENT AND TRANSPORT RATE

The Effect of Evaporating Conditions on Dye Transport

In the studies which follow, the transport into the fruit-body of various dyes was investigated by aseptically introducing 0·25 ml. of a concentrated aqueous solution of the dye into the liquid medium below the mycelium. The principal dyes used were fluorescein (at 0·005 per cent. in the medium) and trypan blue (at 0·01 per cent. in the medium) neither of which showed any evidence of toxicity at these concentrations. Fluorescein-injected fruit-bodies were examined in ultra-violet light, whereby early traces of colouring were more readily detected. Similarly, such specimens were photographed in u.v. light using a chromatic plate and orange filter so that some of the striking contrast noticeable to the eye was reproduced. A similar photographic procedure, but employing light of the visible spectrum, was, for a like reason, used in connexion with trypan-blue-tinted sporophores. The experiments with dyes were usually applied to young fruit-bodies *c.* 10 mm. in length, the stage at which cap formation is about to occur, or has started, if the cultures are grown under conditions of rapid transpiration. The usual rapid- and slow-evaporation conditions were applied (see Methods) at a light intensity (60 f.c.) allowing a full expression of the transpiration effect. Striking and consistent differences in dye ascent were associated with the evaporating conditions.

With rapid evaporation most fruit-bodies show some evidence of the rise of fluorescein into the upper parts of the stipe in 24 hours and after 48 hours it is general that the whole fruit-body and forming cap is tinged yellow and in u.v. light fluoresces brightly. With slow evaporation, on the other hand, ascent is much slower. It is rarely that a slight colouring of the stipe base is visible after 24 hours. After 2 days such fruit-bodies may show some slight evidence of the dye in all parts, but have never been observed to become brightly yellow even after several weeks. Plate Nos. 2 and 3 show the deep injection of the dye into 'dry'- as compared with 'wet'-atmosphere fruit-bodies after 2 and 4 days from the time of introduction of the fluorescein.

With trypan blue an even more marked contrast is obtained. In one day rapidly transpiring fruit-bodies are often blued up to about 7 mm. from the base and the whole fruit-body, looking like a different species, is bright blue in two days. Conversely, in moist air, fruit bodies show no visible ascent of the dye after 3 days except at the extreme base of the stipe and this appears to advance no farther over periods of weeks. The contrast is shown 2 days after the addition of trypan blue in Plate No. 4.

Transfer of a stipe developing under conditions of slow evaporation, in the presence of either dye, to a rapid transpiration state leads to a conspicuous ascent of pigment to all parts of the fruit-body.

Various other dyes were tested. Methylene blue and neutral red gave results very similar to those with trypan blue. Pontamine sky blue 6 BX, however, showed no rise into fruit-bodies after 2 days and at 6 days it was still invisible in sporophores in slow-, and only faintly visible in sporophores in rapid-transpiration conditions.

The Effect of Light Intensity on Dye Transport

Like rapid evaporation, relatively high light intensities promote pileus development in *P. brumalis.* It was, therefore, investigated, using the two intensities 40 and 160 f.c., whether dye ascent was influenced by this factor. There were two experiments, the first at an intermediate evaporation level induced by passing air of 53 per cent. R.H. (conditioned with saturated

$Ca(NO_3)_2$, $4H_2O$) into the experimental chamber at 200 ml./min. and the second with the very slow evaporation conditions described elsewhere in this paper. High evaporation conditions were avoided as likely to swamp any light effect.

In the first experiment dye ascended fairly rapidly and similarly into fruit-bodies at both light intensities. This is a result which might be expected under the fairly vigorous evaporation conditions applied. However, small differences did occur. Thus, in the epileate fruit-bodies in weaker light some stipe apices tended to remain uncoloured, especially in rapidly growing individuals, as though the apex grew at a rate which outstripped the advance of the dye. This was still apparent 10 days after the introduction of the dye (see trypan-blue stained fruit-bodies Plate No. 5) although other, often slowly growing, stipes were completely injected.

In the second experiment no obvious difference was observed between high and low light intensity treatments. Trypan-blue scarcely showed any ascent at all and fluorescein caused a slight all over yellowing of fruit-bodies in the way reported for 'slow' evaporation conditions in previous experiments. It is noteworthy, however, that an exceptional fruit-body which produced a pileus under the influence of the higher light intensity became, unlike other fruit-bodies in the same chamber, fairly deeply injected with fluorescein. Here is a suggestion, recurrent in this work, and discussed further in the next section, that dye ascent may be related to pileus production independently of evaporation. The differences between low- and high-light intensity sporophores in the previous experiment may have a similar explanation.

Primarily it was shown by both tests that light intensity contrasts strongly with evaporating conditions in having a relatively minor effect upon dye ascent. Taken together the experiments with dyes lend strong direct support to the hypothesis that stronger evaporation conditions result in an enhanced rate of translocation of materials towards the fruit-body apex.

3. THE PATH AND MECHANISM OF TRANSPORT IN THE FRUIT-BODY

Attempts to obtain direct evidence by microscopical examination of the location of the dye in injected fruit-bodies gave inconclusive results. In hand-sections the context of the fruit-body had the same coloration as the surface layers. Such sections were teased-out with fine needles and examined microscopically. Although, at low magnifications, individual hyphae often showed a faint coloration, this usually disappeared in the strong light required at the high magnifications necessary to view the interior of hyphae. In no case could a distinction be made between the possibility of the pigment lying within the cell and that of its being adsorbed on the outer surface of the cell wall. Such was the situation using fluorescein, trypan blue, neutral red and in fruit-bodies stained with prussian blue. Prussian blue staining was effected by including saccharated iron oxide in the culture medium and then 'developing, sections of the fruit-body by treatment with ammonium sulphide and potassium ferricyanide.

When sections of trypan-blue-stained fruit-bodies were mounted in water and teased-out, no immediate blueing of the mounting liquid occurred. After an hour or more, however, a blanching of the fungus tissue, especially if from the apex of the fruit-body, was associated with tinting of the mountant. Slices of fluorescein-injected fruit-bodies would instantly stain the water droplet into which they were placed, the hyphae remaining yellowish and fluorescent. Without information concerning the rate at which the dyes might pass the cell membranes and not knowing whether adsorption on hyphal surfaces occurs, these results are only suggestive. An intrahyphal location for trypan blue and perhaps an intra- and inter-hyphal site for fluorescein is one possible interpretation of the facts.

Perhaps the best evidence concerning mechanism and, indirectly, pathway of dye ascent, was obtained in the already described experiments on injection of rapidly transpiring fruit-bodies with trypan blue and fluorescein. Occasionally one of a group of such sporophores would fail to colour like its fellows. Invariably such a fruit-body was proved through further observation to be arrested in growth or very slow growing. This feature is shown clearly on one of the rapid-evaporation group of sporophores photographed 1, 2, and 4 days after the introduction of the dye in Plate Nos. 1, 2 (high-evaporation specimen), and 3 (high evaporation specimen). Here is evidence that transport is not wholly determined by evaporation but is dependent on the biological activities associated with growth. Such a system could be more readily envisaged as entailing an intrahyphal route.

Similar evidence is available from killed fruit-bodies. Groups of fruit-bodies were grown under good conditions for cap development (and hence dye ascent). Light intensity was 80 f.c., temperature 24° C., and a high, though unmeasured, transpiration rate was assured by the introduction of a large amount of anhydrous $CaCl_2$ below the shelves of the glass desiccator chambers in which the fungi grew. When cap formation was starting one group of cultures was killed by the introduction of an open dish containing a few ml. of chloroform into the chamber. Trypan blue and fluorescein were added to the media of some of the cultures in this and control chambers. The usual injection of fruit-bodies occurred in the controls, reaching a maximum visibility in 2 days. In killed fruit-bodies, on the other hand, fluorescein ascended weakly and seldom reached the apex, in a manner reminiscent of dye-treated living sporophores growing under slow evaporation conditions or arrested in growth. Trypan blue entered killed sporophores only at the extreme bases of some stipes. It is true that killed sporophores might differ mechanically from living ones owing, for example, to loss of cell turgor and escape of cell contents; nevertheless the result supports the view that some property of the living cell is necessary for copious dye ascent.

DISCUSSION

The effect of heightened evaporation in causing hastened production of more numerous and larger pilei shown previously (Plunkett, 1956) is now seen to be intimately associated with increased transport rates in the stipe as indicated by accelerated dye ascent and by increased rates of dry matter increment at the apex.

The probable interpretation of these facts is that more rapid evaporation conditions, which increase transpiration (Plunket, loc. cit.), hasten the upward transport stream, thereby bringing, in unit time, greater supplies of its contained solutes to the growing region and thus promoting dry matter and cap production. In such a system concentration of the transport stream as well as flow rate should affect behaviour at the apex. It is, therefore, confirmatory evidence that reduction of medium concentration (and hence probably of the concentration of the translocation stream), without change of evaporating conditions, diminishes cap production.

The high order of the effect of evaporating conditions upon dye ascent and growth at the stipe apex makes it probable that evaporational suction is largely concerned in transport. Thus, the change from 'rapid' to 'slow' evaporating conditions caused a reduction in rate of apical dry matter accumulation of about 75 per cent. The reduction of rates of dye ascent was much greater than that in a number of agarics, where transfer to a saturated atmosphere may only reduce transport rate by about 17 per cent. (Schütte, 1956).

However, growth of *P. brumalis* does occur in air with the almost negligible saturation deficits used in the 'slow' evaporation conditions of the present experiments. It follows that some transport must occur under these conditions, and, indeed, this is shown by the slow accumulation of dry matter at the apex and the transport, albeit very feeble, of dyes into the sporophore. Inexplicably so far, trypan blue is a much poorer indicator of this residual translocation than fluorescein.

In two unrelated experiments odd fruit-bodies which, untypically, produced pilei under slow evaporating conditions showed, in one case, better dye injection and, in the other, greater apical growth-rate than their fellows. It is not wished to attach great weight to so few observations, but in a very preliminary way they seem to suggest that cap formation and moderately rapid transport into the fruit-body may be related, quite apart from evaporating conditions. Again, this might be indicated by the slightly more effective dye ascent into well-illuminated (and hence pileate) sporophores as compared with some dimly illuminated (and hence epileate) sporophores under the same fairly high evaporating conditions. More importance may be attached to the fact that fructifications which are arrested in growth, or killed, show nothing but the feeblest translocation, even in dry air. Thus, it is probable that evaporation plays its dominant role in transport in *P. brumalis* by operating upon some essentially living system, a concomitant of growth, the failure of which limits transport. It is not unreasonable to look to this 'vital' component of translocation for the residual slow transport which occurs in very moist air. Perhaps similar is 'the vital phenomenon linked with the purely physical process of evaporation' postulated in agaric transpiration and hence transport (Schütte, loc. cit.), although there residual transport was greater. A dependence of translocation on the activities of growing cells may also have been present in the agarics, as rapid transport was a juvenile characteristic that declined at maturity.

In the present experiments, unlike those with the agarics, the sporophores were attached to the mycelium so that 'mycelium pressure', if present, was free to operate. It might represent the vital component of translocation, although the case of the agarics makes it clear that this is not necessarily so.

In summary, translocation in *P. brumalis* probably involves an indispensable vital component although the movement is due mainly to evaporation, whereas in agarics, under Schütte's conditions, the vital component (which is not mycelium pressure) dominates the process and evaporation is subsidiary and dispensable.

The relatively greater importance of evaporating conditions for translocation in *P. brumalis* harmonizes with the fact that in this fungus [and probably in another polypore, *Ganoderma applanatum* (Hopp, 1938)] evaporation rates have a formative influence upon pileus development, whereas in the only agarics investigated [*Collybia velutipes* (Plunkett 1956) and *Coprinus lagopus* (Borriss, 1934)] they have no such effects. It is further relevant to this point that the absolute rates of transport in Schütte's agarics were very high, often of the order of 10 cm./hr., which contrasts strikingly with the gradual injection of *P. brumalis* sporophores even under fairly rapid evaporating conditions.

The question of the path of transport in the sporophores of *P. brumalis* and other hymenomycetes is still open, but in view of the further indication of the activities of living hyphae in translocation an intrahyphal channel becomes more likely.

ACKNOWLEDGEMENT

As on previous occasions I am much indebted to Professor C. T. Ingold for his encouragement and for critical reading of the manuscript.

LITERATURE CITED

BONNER, J. T., KANE, K. K., and LEVEY, R. H., 1956: Studies on the mechanics of growth in the common mushroom, *Agaricus campestris*. Mycologia, **48**, (1), 13–19.

BORRISS, H., 1934: Über den Einfluß äußerer Faktoren an Wachstum und Entwicklung der Fruchtkörper von *Coprinus lagopus*. Planta, **22**, 644–84.

BULLER, A. H. R., 1933: Researches in Fungi, Vol. **5**, London.

HOPP, H., 1938: Sporophore Formation by *Fomes applanatus* in Culture. Phytopathology, **28**, 356–60.

MADELIN, M. F., 1956 *a*: Studies on the Nutrition of *Coprinus lagopus* Fr., especially as affecting fruiting. Ann. Bot., N.S., Lond., **20** (78), 307–30.

—— 1956 *b*: The Influence of Light and Temperature on Fruiting of *Coprinus lagopus* Fr. in pure Culture. Ibid. (79), 467–80.

PLUNKETT, B. E., 1951: Some Aspects of the Physiology of Fruit-body production in the Hymenomycetes. Ph.D. thesis, University of London.

—— 1956: The Influence of Factors of the Aeration Complex and Light upon Fruit-body Form in Pure Cultures of an Agaric and a Polypore. Ann. Bot., N.S., Lond., **20** (80), 563–86.

SCHÜTTE, K. H., 1956: Translocation in the fungi. New Phytol. **55**, (2), 164–82.

VODERBERG, K., 1950: Die Abhängigkeit der Fruchtkörperentwicklung bei *Coprinus lagopus* von inneren und äußeren Faktoren. Planta, **37**, 612–25.

WAHRLICH, W., 1893: Zur Anatomie der Zelle bei Pilzen und Fadenalgen. Scripta Botanica Horti Universitatis Imp. Petropolitanae, **4**, 101–55 (cited in detail in Buller, 1953).

DESCRIPTION OF PLATE

The smallest divisions of the scale lines on the plate all represent 1 cm.

Nos. 1, 2, 3. Photographed in u.v. light using a chromatic plate and orange filter.

Nos. 4, 5. Photographed in light from tungsten filament lamps using a chromatic plate and orange filter.

No. 6. Photographed as 4 and 5 but without filter.

FIG. 1. Young fruit-bodies lightly injected with fluorescein 24 hours after the introduction of the dye into the medium, under 'rapid' evaporating conditions.

FIG. 2. Young fruit-bodies 48 hours after addition of fluorescein. Under, left: 'rapid' evaporating conditions (the same group as in Fig. 1), right: 'slow' evaporation. Note greater fluorescence of 'rapid' evaporation specimen.

FIG. 3. The same fruit bodies and conditions as in Fig. 2, after 96 hours.

(In Figs. 2 and 3 a control culture without fluorescein lies between the two treated ones. A small panel, more lightly printed, shows its presence, especially in 3.)

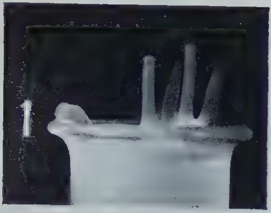
The group of 4 fruit-bodies growing under 'rapid' evaporation conditions in Figs. 1, 2, and 3 show that fluorescein injection occurs quickly only if the sporophore is growing.

FIG. 4. Young sporophores, 48 hours after addition of trypan blue. Left: 'slow', right: 'rapid' evaporation (dye injection only visible in the latter).

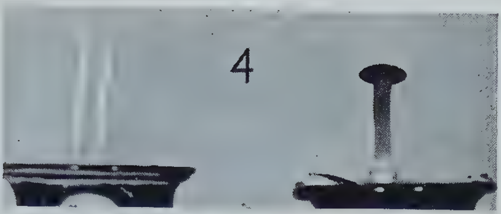
FIG. 5. Trypan-blue-treated fruit-bodies after 10 days. Left to right: first; control (no dye), second and third; high light intensity (160 f.c.), deep dye injection, fourth and fifth; 40 f.c., some fruit-bodies deeply stained, others (fast growing) unstained at apices.

FIG. 6. Crop on 5 per cent. malt extract; transferred prior to cap formation from 'slow' evaporation and low light intensity conditions to more rapid evaporation and higher light intensity.

Note increase in diameter about half way up the stipes in the three central sporophores.

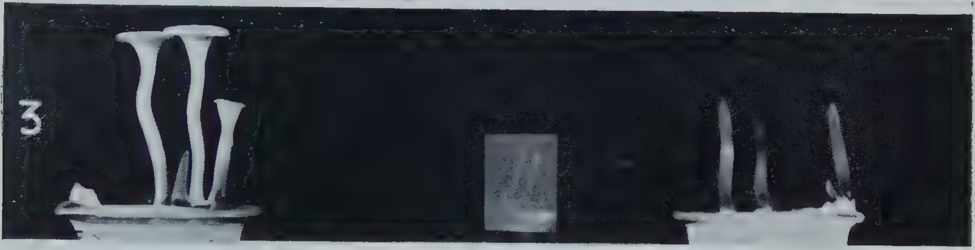


1

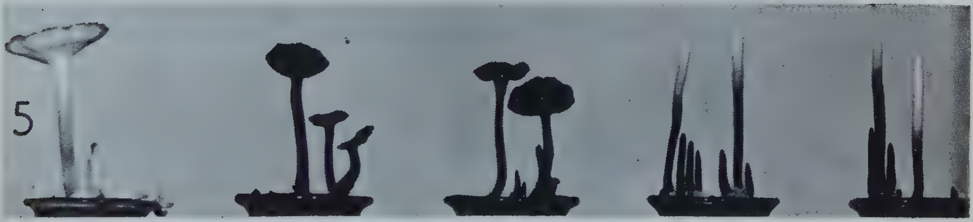


4

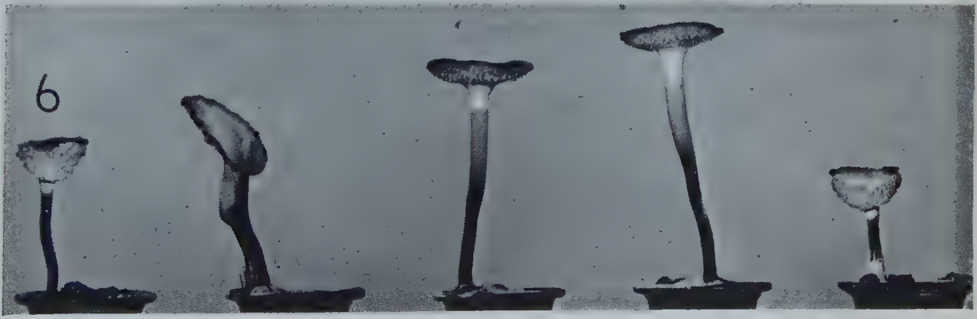
2



3



5



6

B. E. PLUNKETT

Effect of Removal of a Part of the Root System on the Subsequent Growth of the Root and Shoot

BY

E. C. HUMPHRIES

(*Rothamsted Experimental Station*)

With four Figures in the Text

ABSTRACT

Removal of up to 50 per cent. of the roots of barley and rye has no effect on the growth-rate of the root which is the same as in the intact plant. In contrast the growth-rate of the shoot decreases as more roots are removed. When more than 50 per cent. of the roots are removed, root growth declines but not so rapidly as that of the shoot. Similar results are obtained by the removal of lateral roots of tomato but root growth begins to decline when 40 per cent. of the lateral roots are removed.

The uptake of potassium by barley plants with proportions of the root system excised is closely proportional to the dry-matter increase when the nutrient supply is not limiting. In conditions of low nutrition the potassium uptake is less than the dry-matter increase.

INTRODUCTION

RICHARDSON (1953) has shown that root growth of *Acer* spp. is dependent upon conditions for photosynthesis. Removal of a part of the shoot or variation in the environment of the shoot such as a change in light intensity or temperature has an almost immediate effect on root growth-rate. It is assumed that this is caused by a change in the availability of the products of photosynthesis. It is also known that removal of a part of the root system will lower the growth-rate of the shoot, but on the other hand, there is little information about the effect of removal of a part of the root system on the subsequent growth of the roots. A number of simple experiments have been performed on barley, rye, and tomato to investigate this point.

The plants were grown in solution culture usually in tanks of 12 litres capacity but in some experiments the plants were grown in bottles. After several weeks' growth, plants had different fractions of their root systems removed as estimated by eye. The actual amount of root system excised was estimated by comparing the dry weight of the part removed with that of the root system of whole plants harvested at the same time. After a further growth period of 2 to 3 weeks the plants were harvested and the increase in growth (expressed as dry matter) of the roots and shoots estimated.

EXPERIMENTAL RESULTS

Experiment 1 (Barley var. 'Herta'). The plants were grown in tanks of culture solution at the rate of 48 plants per 12 litres of solution which consisted of either half-strength (High NPK) or one-sixth strength Hoagland solution

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

(Low NPK). After 23 days' growth varying numbers of adventitious roots were removed by cutting as close to the point of attachment to the stem as possible. A group of six plants constituted a sample. The aim was to remove approximately one-third or two-thirds of the total root system. The amounts actually removed averaged about 35 and 54 per cent. by weight, respectively. The plants were allowed to grow for a further 11 days. The result have been expressed as regression lines relating increase in dry matter to the percentage of root remaining on individual samples at the time of operation (see *A* in Fig. 1). The increment of root dry matter was the same regardless of the amount of root removed and the regression coefficients are not significant. Moreover, there was little difference in the increase in weight of roots at the two levels of nutrition. Thus, removing up to 50 per cent. of the root system had no effect on the growth-rate of the roots compared with that of roots on the intact plant. There was, however, a marked effect on the growth of the shoots, shown by a significant regression of increase in dry matter on the percentage of root remaining. (For the high NPK treatment the regression is $+0\cdot013\pm0\cdot006$ and for the low NPK $+0\cdot011\pm0\cdot0035$ in g. dry matter per 1 per cent. of root system.) The dry matter of shoots declined at much the same rate at the two nutritional levels but there was a significant difference between the means of the two treatments reflecting the different nutrition. When the results are expressed as relative growth-rates (log. initial dry weight–log. final dry weight) the difference between the behaviour of roots and shoots is even more striking. The relative growth-rate of the root increases as the amount of root removed increases while that of the shoot declines (*A* in Fig. 1). All the regression coefficients are significant.

In order to see the effect of root removal on nutrient uptake in this experiment, the potassium contents of the roots and shoots were measured. Table 1 shows the uptake of potassium expressed as a percentage of the control compared with the total dry-matter increases also expressed as a percentage of the control.

TABLE 1

Dry-matter Increase and Potassium Uptake of Barley Plants with part of the Root System Removed

High NPK			Low NPK		
% roots remaining	Total dry-matter increase % of controls	Total K uptake % of controls	% roots remaining	Total dry-matter increase % of controls	Total K uptake % of controls
65	78	77	62	77	65
52	68	66	47	75	65

At the higher level of nutrition K uptake is closely similar to the increase in dry matter but in the low NPK treatment the K uptake is relatively less than the dry-matter increase. There was no indication that the new roots produced after excision increased the rate of potassium uptake.

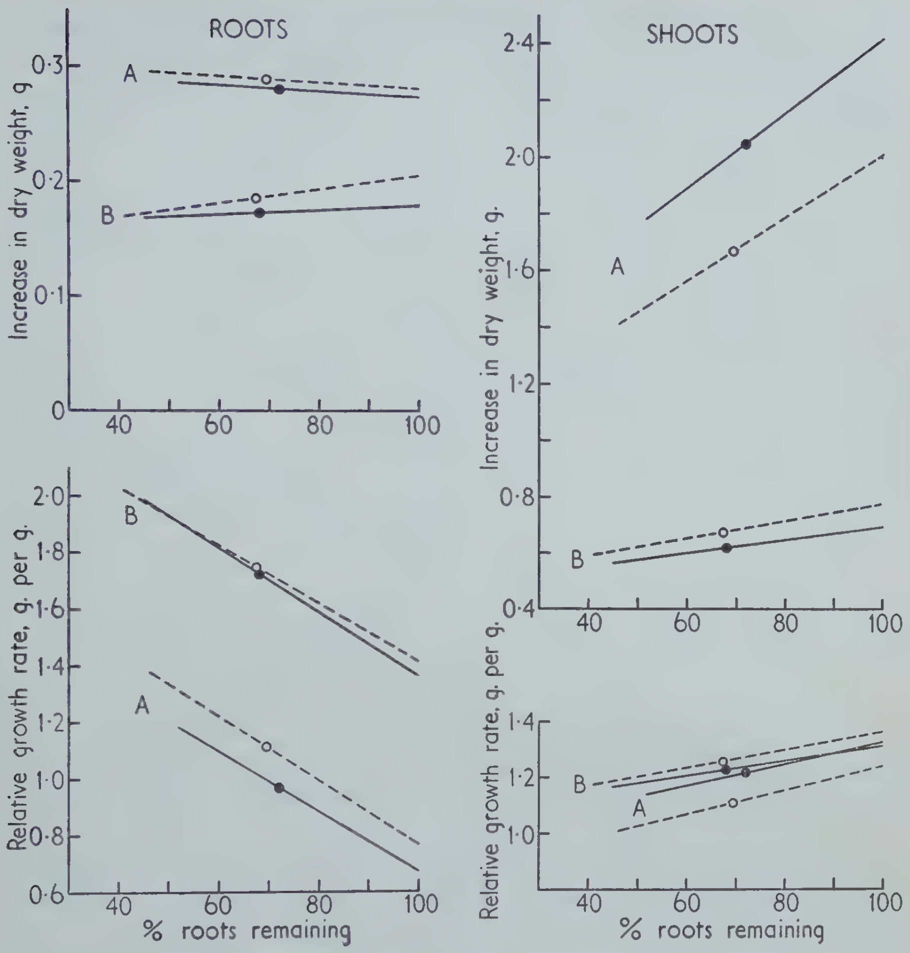


FIG. 1. Absolute and relative growth-rate of roots and shoots of barley after varying proportions of roots were excised. ——— High NPK, - - - - Low NPK

A—roots cut off after 23 days' growth with 11 days' subsequent growth,
B—roots cut off after 14 days' growth with 13 days' subsequent growth.

Experiment 2 (Barley var. 'Herta'). This was a similar experiment to the foregoing but the plants were sampled at a younger stage. Root excision took place after 14 days' growth and the plants were harvested after a further 13 days' growth. As in the previous experiment, there was no detectable influence of the amount of root removed on subsequent root growth-rate (see B in Fig. 1). The amount of growth compared with Exp. 1 was smaller—in accord with the shorter growing time before root excision. The decrease in top growth was also less, but the regression coefficients were significant (High NPK $+0.0024\pm0.0007$; Low NPK $+0.0031\pm0.0011$). The relative growth-rates of the roots were similar to those in the first experiment but with a higher mean value. The relative growth-rates of the shoots were also similar to those of the first experiment.

Similar results to those described in Experiments 1 and 2 were obtained with rye.

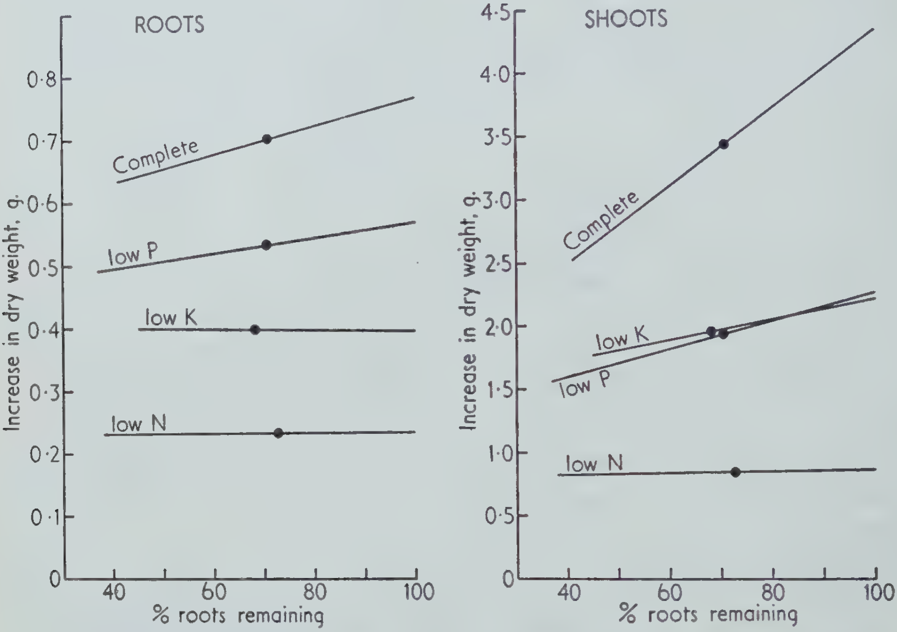


FIG. 2. Change in absolute growth-rate of roots and shoots of barley, grown under different nutrient conditions after varying proportions of roots were excised.

Experiment 3 (Barley var. 'Herta'). In this experiment the subsequent growth of roots and shoots after root excision was investigated under conditions of low N, P, or K compared with complete nutrition. The rates of N, P, or K supply were reduced to one-sixth of those in the complete solution which, as before, was half-strength Hoagland solution. The plants were 27 days old at time of root excision and had 14 days' subsequent growth. In all treatments root excision had no effect on root growth, all regression coefficients being not significantly different from zero (Fig. 2), but the amount of growth made by the roots subsequent to excision was a reflection of the nutrition received. The roots in the complete culture solution made most growth, while those in the low nitrogen made least. The effect of root excision on shoot growth was most marked in the plants grown in complete solution, while the reduction in growth in the low P and low K treatments was similar. In the low-nitrogen treatments there was no reduction in the growth of the shoots as a result of root excision and it is suggested that the low level of nitrogen was already limiting the growth-rate before root excision took place.

Experiment 4 (Barley var. 'Herta'). The results of the previous experiments indicate that when up to 50 per cent. of the roots is removed, the subsequent growth-rate of the root system is the same as that of the intact plant. It is of interest to see the effect of removing greater proportions of the root system than in the first three experiments. The plants for this experiment were grown in pint bottles with two plants in each; they were 20 days old at the time of root excision, and had 17 days' subsequent growth. The results are shown in Fig. 3. As before, with 50 per cent. or more of the roots remaining there was no significant change in the growth-rate of the roots but with less than 50 per cent. of the roots remaining the root growth declined until with all roots removed growth was reduced to 56 per cent. of the control. The effect on shoot growth was more marked and there was a continuous decline in growth as the amount of root removed increased. Removal of all roots reduced the shoot growth to 42 per cent. of the control.

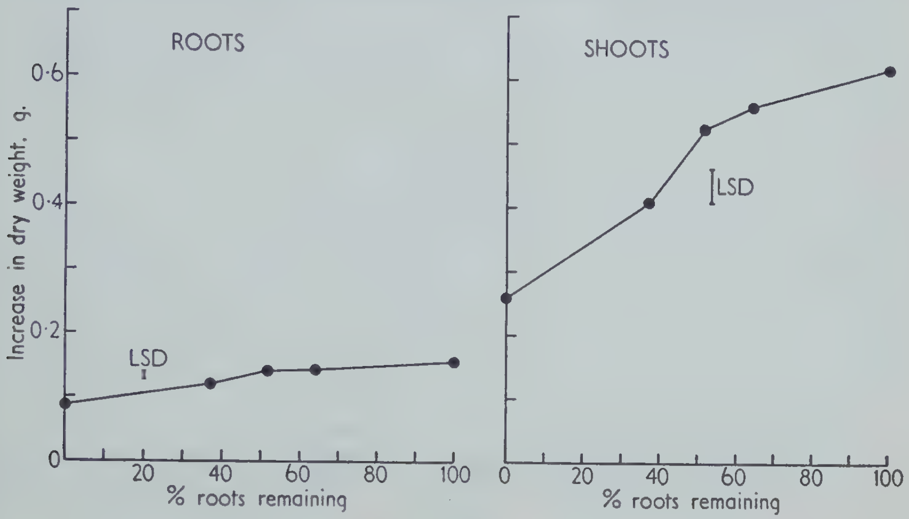


FIG. 3. Changes in absolute growth-rate of roots and shoots of barley after varying proportions of roots were excised.

Experiment 5 (Tomato var. 'Sunrise'). Seeds were germinated in sand and transplanted to pint bottles of complete nutrient solution (one plant per bottle). After 39 days' growth varying proportions of the lateral roots were excised leaving the tap-root intact. The plants were harvested 14 days later. The increments in dry matter of shoots and roots have been plotted in Fig. 4A against the percentage of lateral roots remaining. The tap root accounted for 45 per cent. of the whole root system at the time of excision. There was no significant change in dry-matter increase of the root with 60 per cent. or more of the laterals remaining (equivalent to 78 per cent. of the whole root system), while the root growth with all laterals removed was 54 per cent. of the control. There was no significant reduction of shoot growth with 74 per cent. of laterals remaining, but as more laterals were removed a sharp reduction of shoot growth occurred. Removing all laterals reduced top growth to 46 per cent. In Fig. 4B the final dry weights of leaves and stems are shown. The dry weight of the stem is not affected until 42 per cent. of the laterals have been removed. Removal of all laterals reduced stem growth to 60 per cent. The effect of root excision had a rather greater effect on leaf dry weight which was reduced to 53 per cent. when all laterals were removed. The number of leaves was reduced in the groups with 0 and 41 per cent. laterals remaining but this reduction was not significant. The number of visible lateral shoots, however, was significantly reduced in these two groups (1·9 per plant compared with 3·1 in the control).

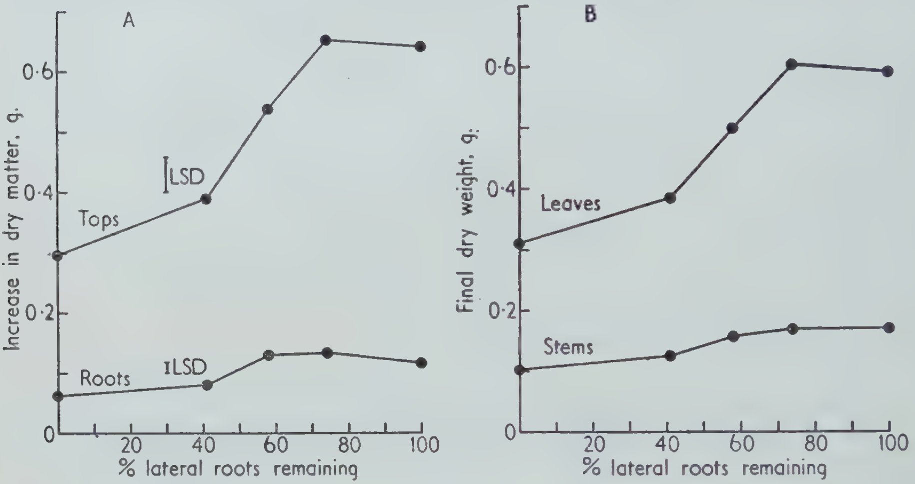


FIG. 4.

A—Changes in absolute growth-rate of roots and shoots of tomato after varying proportions of lateral roots were excised.

B—Total dry matter in leaves and stems from germination.

DISCUSSION

Removal of part or the whole of the root in barley, rye, and tomato leads to a differential effect on the growth-rate of shoot and root. In barley and rye removal of up to 50 per cent. of the roots has no effect on the growth-rate of the root which is the same as in the intact plant. The growth-rate of the shoot decreases with increasing amount of root removed. This is apparently due to the supply of nutrients to the shoot being retarded by the reduced root system while the supply to the root is still adequate. At the same time the supply of carbohydrate to the root from the shoot is apparently not immediately limiting. It is suggested that when the root system is reduced below 50 per cent., the mineral supply even to the root system becomes limiting and so the growth-rate of the root declines. It is apparent from Fig. 3 that when less than 50 per cent. of the root system remains shoot growth shows a greater decline than when 50 per cent. remains or in the intact plant. A similar state of affairs exists in the tomato, but cannot be compared directly with barley or rye because of the differentiation of the root system into a tap root with laterals.

It is well known that the top/root ratio is extremely variable within a species and the foregoing experiments suggest an explanation. The roots and shoots are in competition for minerals absorbed by the root and carbohydrates manufactured by the shoot. When the mineral supply is limiting, the root will grow relatively more than the shoot, while a reduction of photosynthetic products will have an immediate effect on root growth.

LITERATURE CITED

RICHARDSON, S. D., 1953 *a*: Studies of Root Growth in *Acer saccharinum* L. 1. The Relation between Root Growth and Photosynthesis. Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam C. **56,** 185.

—— 1953 *b*: Studies of Root Growth in *Acer saccharinum* L. 2. Factors affecting Root Growth when Photosynthesis is Curtailed. Ibid. 346.

An Analysis of the Factors Controlling the Germination of the Seed of the Oil Palm, *Elaeis guineensis* (Jacq.)

BY

G. HUSSEY*

(*West African Institute for Oil Palm Research, Benin City, Nigeria and Research Institute of Plant Physiology, Imperial College of Science and Technology, London*)

With Two Plates and six Figures in the Text

ABSTRACT

Under natural conditions in West Africa at the average ground temperatures of 25–30° C., oil-palm seed germinates sporadically over a period of years, but with continuous heat treatment (38–40° C.) 50 per cent. germination of either nuts or extracted kernels occurs within a few months.

The kernel comprises a small cylindrical embryo embedded in a mass of oily endosperm which in turn is surrounded by a thick testa. At germination the testa and layer of endosperm covering the distal part of the embryo are forced out as a disc-like operculum which is already demarcated in the ungerminated kernel by a circular abscission zone in the endosperm cells.

The excised embryo is non-dormant and at 30° C. starts to elongate within 24 hours, either on moist filter paper or while still in contact with the endosperm.

At 30° C. removal of the operculum is followed by sluggish emergence of the embryo in air; emergence at the normal rate occurs in pure oxygen, or in air if a long period at high temperature precedes de-operculation. Fresh intact kernels remain ungerminated for at least 6 months at 30° C., either in air or in pure oxygen.

At high temperature (40° C.) intact kernels germinate faster in pure oxygen than in air; at 30° C. the same applies only after previous treatment at high temperature. This accelerating effect of oxygen is shown to be dependent upon the progress of slow processes stimulated by high temperature.

It is postulated that germination of oil-palm seed is dependent upon a minimal threshold concentration of oxygen in the embryo and that during dormancy this minimal level progressively decreases at a rate depending on the temperature.

INTRODUCTION

THE slow and erratic germination of oil-palm seed has been recognized as a serious practical problem since the middle of the nineteenth century, when oil-palm cultivation was first started in the Far East. In the absence of vegetative propagation, the establishment of oil-palm plantations is dependent upon the successful germination of selected seed. The problem is of considerable importance in West Africa where nursery and field planting are restricted to the few weeks at the beginning of the annual wet season. In these circumstances rapid and controlled germination is highly desirable so that sufficient numbers of uniform seedlings are available when required.

During the past 30 years, both in Africa and in the Far East, the problem has been tackled almost exclusively from the practical angle and the causes

---

* Present Address: John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

of erratic germination are not well understood. This paper describes an analysis of the main factors affecting dormancy in the oil-palm seed, recently undertaken at the West African Institute for Oil Palm Research (W.A.I.F.O.R.), Nigeria. Results of physiological interest only are reported here, but a full account of the practical applications that follow will be published shortly in the Journal of W.A.I.F.O.R. Before the experimental work is described, it will be necessary to give an account of the morphology and anatomy of the oil-palm seed, together with a summary of its general germination behaviour.

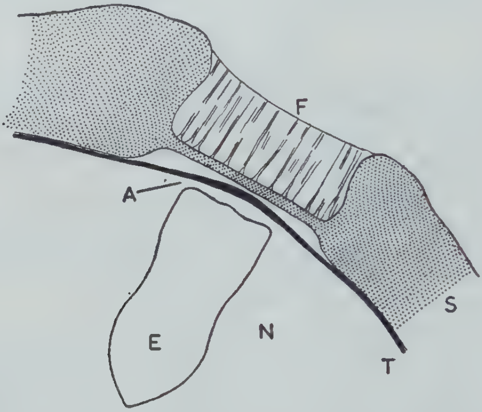


Fig. 1. Diagrammatic L.S. of part of a *tenera* nut showing embryo and germ pore. E, embryo; N, endosperm; T, testa; S, shell; F, fibre plug cemented together at the base to form a plate-like structure which merges with the shell; A, region of abscission zone. (Compare Plate 1, Fig. 3.)

STRUCTURE AND GERMINATION OF THE SEED

Morphology. Oil-palm seedlings are germinated from 'palm nuts' which consist of a stony endocarp, or shell, enclosing a kernel which contains a small embryo. Although naked kernels will also germinate and produce seedlings, they are susceptible to infection by micro-organisms and cannot therefore be used in practice.

The oil-palm fruit, which is a drupe, develops from a trilocular ovary in which each separate carpel bears a single ovule. Usually one or two ovules abort, so that approximately 75 per cent. of nuts are single seeded, i.e. contain one kernel only, 23 per cent. are two-seeded, and the remaining 2 per cent. three-seeded. There are both thick- and thin-shelled forms (*dura* and *tenera* respectively). The general morphology and anatomy of the seed as described by Yampolsky (1922) lacks details of the structures in the immediate vicinity of the embryo and the actual process of germination. The germ pore, shown diagrammatically in longitudinal section (L.S.), in Fig. 1, is wider than the embryo which lies beneath it. Plate 1, Fig. 3 is a photograph of a *tenera* nut split longitudinally and shows the germ pore and embryo *in situ*.

The germ pore is blocked by a plug of fibres which are cemented together at the base to form a plate-like structure continuous with the shell at its periphery. The thickness of this structure varies from between 0·2 mm. and 0·3 mm. irrespective of the general thickness of the shell. At germination the plug of fibres is ruptured and forced out of the germ pore.

The kernel consists of a hard mass of white oily endosperm tissue surrounded by the testa, a dark coloured integument composed of a multicellular layer of small quadrangular cells with dense tannoid contents. The testa varies in thickness from 0·05 mm. to 0·15 mm. and is thicker where it covers the distal part of the embryo.

The embryo is a small cylindrical body approximately 3 mm. in length embedded in the endosperm near the apex of the kernel. A slight constriction divides the embryo into two parts, the haustorium, sometimes called the cotyledon, and the distal end containing the tigellum. The haustorium is lighter in colour than the rest of the embryo and is marked with shallow longitudinal furrows. After germination, when the distal part of the embryo has emerged from the germ pore, the haustorium grows and absorbs the endosperm, forming a spongy mass of tissue which eventually fills the entire kernel.

The distal part of the embryo is separated from the testa by a thin layer of 4 to 6 endosperm cells together approximately 0·1 mm. thick.

Germination begins abruptly by elongation of the embryo and simultaneous rupture of the tissues covering its distal end; the elongating embryo then forces its way through the germ pore. When separated kernels are allowed to germinate, the testa and layer of endosperm covering the embryo are forced out in the form of a disc (Plate 2, Fig. 5) which usually adheres to the elongating embryo (Plate 2, Fig. 6). In the entire nut, the tissues comprising this disc lie between the distal part of the embryo and the plug of fibres blocking the germ pore in the shell, and on germination both these structures, i.e. disc and fibre plug, are forced out together and generally remain in contact. Yampolski distinguishes an operculum, which he describes as being 'kept rigidly in place on the one side by the embryo and on the other side by the fibres and fleshy tissue of the pericarps which fill up the germ canal'. It is clear from Yampolsky's description and from the text diagram on page 149 of his paper, that what he designates as the operculum comprises the two distinct structures described here; viz. the plug of fibres cemented at the base into a plate-like structure continuous with the inner surface of the shell, and the kernel tissues immediately below, which become forced out in the form of a disc. In the ungerminated kernel the disc-like structure is already demarcated by a circular band of endosperm cells which have smaller dimensions than those of the adjacent cells of the endosperm. There is, however, no corresponding differentiated zone apparent in the cells of the testa. This is shown clearly in Plate 2, Fig. 7, which is a photomicrograph of a L.S. of an ungerminated *tenera* kernel in the region of the embryo. The accompanying photomicrograph (Plate 2, Fig. 8) shows a similar L.S. of a kernel about to germinate, the drag of the microtome knife having been sufficient to rupture the endosperm along this line of cells. This type of fracture was found to occur consistently only in sections of ungerminated kernels that had been soaked for some time at high temperature and were therefore about to germinate. Examination of hand sections of just-germinated kernels showed that rupture of the endosperm tissue normally takes place in this region. Thus in the ungerminated kernel the disc-like structure is differentiated as a distinct organ with an abscission zone separating it from the rest of the endosperm. It would seem more appropriate to apply the term operculum to this organ alone, as the plug of fibres is a separate structure belonging to the pericarp tissues.

Once germination has started, further growth of the embryo proceeds unhindered. After emerging from the germ pore, the embryo swells to form a bulbous structure from which the radicle and plumule arise.

Germination. Under natural conditions in West Africa, oil-palm seed germinates very slowly and sporadically over a period of years, and apart from seed eaten or destroyed by animals, only a negligible proportion produces seedlings. In open protected seed-beds in West Africa where average ground temperatures do not usually exceed 30° C., less than half of the seed may germinate within 12 months, but in other parts of the world, especially the Far East where higher average ground temperatures occur, up to 80 per cent. may germinate after a period of 6 to 8 months (Bunting *et al.*, 1927). It has been recognized for some time that the optimum temperature for germination is fairly high, in the region of 35–40° C., and because of the lower average temperatures experienced in West Africa, much empirical work has been concerned with the artificial provision of heat, both as continuous application and for specific periods. From the results of these experiments it has become clear that constant high temperature is necessary and that short periods at high temperature, the so called pre-heat treatments, are ineffective (Milsum, 1927; Bunting *et al.*, 1927, 1934; Beirnaert, 1936; Toovey, 1937). Preliminary periods at low temperature do not stimulate germination and, if prolonged, result in the death of the seed (Galt, 1952). General experience over the last 20 years has shown that by sowing seed in boxes of moist charcoal maintained at constant high temperature, germination of up to 80 per cent. may be obtained after 4 to 8 months but much lower totals, in the region of 50 per cent. or less, have been more usual.

The optimum temperature has been considered to lie between 36 and 40° C. Vanderweyen (1952) recommends a temperature range of 35–40° C. and claims that the optimum for *tenera* seed is 37° C. Henry (1951) claims that the optimum is 38° C. although he gives no results to support this conclusion. Ferwerda (1956) found little difference in the speed of germination over the temperature range 37–40° C. but did not test temperatures in excess of 40° C. No critical experiments using a wide range of temperatures have been carried out.

In 1951 Henry, working in Dahomey, showed that germination at 38° C.

may be slightly accelerated if the temperature is occasionally lowered to 25–28° C. for short periods. The maximum acceleration was found to occur when the temperature was lowered for 24 hours once every 2 weeks. Henry's results show that cooling during the first 2 months at high temperature has little or no accelerating effect and it is not until after this time, when the first germinations occur, that the cooling becomes effective.

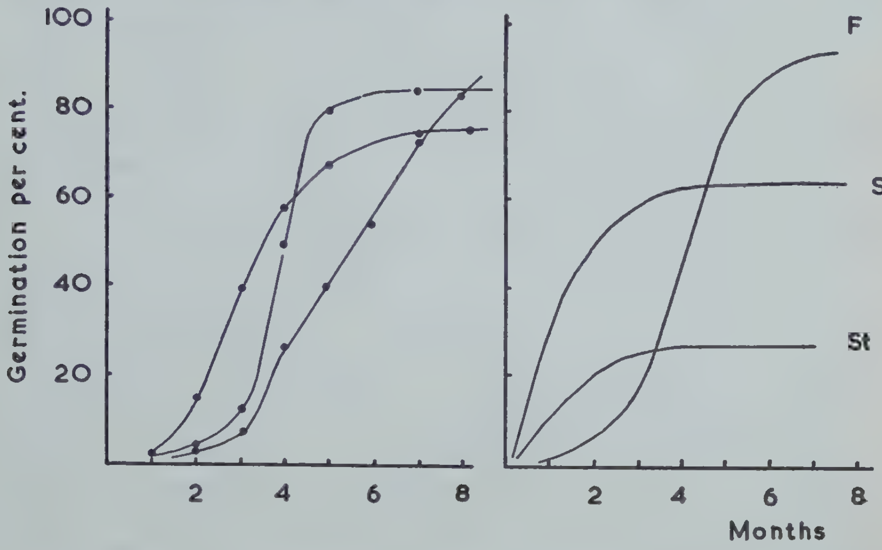


Fig. 2. Fig. 3.

Figs. 2 and 3. Fig. 2 Experimental curves for batches of fresh nuts germinated at constant high temperature (37–40° C.); Fig. 3 Ideal germination curves for fresh seed (F) and for stored seed (St).

There have been numerous attempts to pre-treat seed with the aim of accelerating subsequent germination. These have included soaking in water, acids, alkalis, or solutions of growth substances, cracking the seed, and, as mentioned above, pre-heating or pre-chilling for various periods. None of these treatments has had any significant effect on germination. Many types of media have been tried for germinating seed, including sand, loam, sawdust, compost, and charcoal, both alone and in various combinations. Powdered charcoal has proved to be superior to all other media.

From 1940 to 1951 a number of storage experiments have been carried out at the West African Institute for Oil Palm Research. The germination of freshly harvested seed has been compared with the germination of seed sampled from the same batch stored under different conditions for varying periods. Freshly harvested seed germinated at constant high temperature typically shows an S-shaped curve when the running total germination is plotted against time. Some experimental curves are shown in Fig. 2. The dormant period is seen to be extremely variable as between individuals, and the average time to germination (i.e. attainment of the 50 per cent. level) between batches of seed may differ by several months. Seed stored for periods of up to 12–18 months is generally much less viable, but germination is faster. The germination curves obtained are of type St shown in Fig. 3; there is no longer any delay in germination which begins at a high rate. Although no critical work on storage of oil-palm seed has been carried out, there is some evidence that these changes in germination behaviour are induced more quickly during damp than during dry storage.

Fresh seed only was used in the experiments reported in this paper.

MATERIALS AND METHODS

This investigation was concerned with the effects of temperature, oxygen, and moisture on the length of the dormant period of nuts and kernels, and with the effects of various factors on the first stage of germination when the embryo begins to grow and to force its way through the germ pore.

Since the dormant period is usually of such long duration, experiments with nuts and kernels, if allowed to run their full course, may occupy periods of up to 6 months or more. Facilities for carrying out germination tests under controlled conditions in the laboratory were limited. It was therefore decided to restrict experiments with nuts and kernels to periods of 2 or 3 months. In such short-term experiments the controls and some of the treatments germinated only to a limited extent, and it was therefore possible to compare the effects of treatments on a qualitative or semi-quantitative basis only. In the experiments with nuts and kernels the replication per treatment consisted of one or two flasks each containing 100 seeds. In most of the experiments the effects of one or two factors were tested over a range of different levels and the germination curves obtained (by plotting running total percentage germination against time) were generally smooth and did not overlap with those of other treatments.

Throughout this investigation seed of the *tenera* form only was used as these take up less space than the thicker-shelled *dura* and are more easily cracked. Each sample of seed for experimental use was selected at random from up to 50 bunches harvested simultaneously.

Kernels were extracted from the nuts by careful cracking in a vice and approximately 70 per cent. were extracted in a sound condition. Each of the kernels was examined under a hand lens and rejected if the slightest injury to the testa was visible. Soaking tests showed that when in a dry condition, both nuts and kernels absorb water rather slowly at temperatures of 25–30° C. Kernels were first soaked in sterile water for 5 days at a temperature of 30° C. Germination tests were carried out in litre conical flasks or 16 oz. jars, which were placed in incubators. Where normal atmospheric oxygen tension was required, the flasks or jars were covered with thin polythene sheeting which allows respiratory exchange of oxygen and CO_2 while minimizing loss of moisture. Increased oxygen tensions were obtained in sealed flasks by flushing with oxygen or mixtures of air and oxygen once every 24 or 48 hours. It was found to be of no consequence whether the flasks were flushed out every

6, 12, or 72 hours provided tubes of soda lime were included in the flasks to absorb accumulated CO_2. Surface contamination of kernels during the course of experiments was reduced to a workable minimum by regular sterilizing with 0·1 per cent. aqueous solution of 8-hydroxyquinoline sulphate and occasionally with 0·1 per cent. solution of mercuric chloride in 10 per cent. alcohol. (During sterilization the kernels had approximately 60 seconds' contact with the sterilizing solutions which were immediately washed away with sterile water.) Kernels that became contaminated internally were discarded. In calculating the percentage germinations the overall loss which averaged 10–15 per cent. was taken into account. The data presented below consist of initial rates of extension growth of embryos, and running total percentages of germination of kernels.

Experiments with Excised Embryos

In the first place it was necessary to establish whether or not the embryo itself shows any dormancy.

Germination of excised embryos on moist filter paper. Embryos excised from freshly soaked kernels were placed on moist filter paper in Petri-dishes under sterile conditions. Preliminary tests were carried out at room temperature which averaged 28° C. Slight elongation and swelling occurred within 24 hours and continued for a period of up to 8 days when the embryos had elongated approximately 1 mm. (Plate 1, Fig. 1). Sections of the elongated embryos showed that both cell division and extension growth had taken place throughout the embryo. Although the rate of extension growth of these excised embryos was a good deal slower than that of embryos that germinate normally in intact seed, it was clear that the excised embryo *starts* to germinate within 24 hours. The slightest elongation of the embryo *in situ* will cause rupture of the operculum and once this has taken place germination continues unhindered. It was therefore concluded that the isolated embryo is non-dormant.

To test the effect of temperature on the germination of excised embryos, the experiment just described was repeated using 4 different temperatures. The results are presented in Table 1 which shows the mean increments in length of 20 embryos per temperature treatment after 4 and 8 days. Most of the extension growth occurred during the first 6 days and the length increments after 8 days represent the final elongation. Elongation at 30° C. was similar to that previously found at 28° C., but at 25° C. elongation was very slight. At the higher temperatures, 36° and 40° C., elongation was more rapid but there were no significant differences in the final lengths.

Germination of excised embryos on nutrient media. Excised embryos germinating on moist filter paper are dependent upon the limited amount of food reserves available in the embryo itself. A few tests were carried out using simple nutrient media. When cultured on White's medium (2 per cent. sucrose agar with inorganic salts only) excised embryos began to germinate slowly at rates similar to those already described for embryos on moist filter paper. This rather sluggish growth continued for several weeks, after which miniature seedlings 2–3 cm. in length (Plate 1, Fig. 2) were formed. In these seedlings, the haustorium always failed to develop and turned a dark brown colour. Seedlings that developed roots did not produce shoots and vice versa. Normal growth of the seedling would therefore appear to be dependent on growth factors supplied by the endosperm in addition to salts and sugar.

TABLE 1

The Effect of Temperature on the Extension Growth of Embryos Excised from Fresh tenera *Kernels Placed on Moist Filter Paper*

Original length of embryo approximately 3 mm.

	Increment in length in millimetres	
		Replication 25
Temperature	After 4 days	After 8 days
25° C.	0·07±0·005	0·15±0·03
30° C.	0·33±0·10	1·06±0·15
36° C.	0·66±0·07	1·69±0·23
40° C.	0·60±0·07	1·05±0·16

Viability of Excised embryos. In all tests with excised embryos the percentage showing elongation always exceeded 95; the rare failures appeared to be caused by bacterial contamination. The individual rates of extension growth, however, were rather variable. The mean increment in length after 4 days at 30° C. averaged 0·4 mm. with a standard error of ±0·10–0·20 mm. and extreme increment values of 0·05–1·0 mm.

EXPERIMENTS WITH WHOLE KERNELS

Although naked kernels are never germinated in practice, several workers (Rutgers, 1922; Galt, 1952; Ferwerda, 1956) have reported tests in which kernels were germinated. These tests have shown that kernels germinate very slowly as do nuts, and require high temperature; removal of the shell does not reduce the long dormant period.

Effect of temperature and oxygen tension. Since lack of oxygen is known to delay the germination of some seeds it was decided to test the effect of increased oxygen tensions. The food reserves, both in the embryo and in the endosperm, consist mainly of oil with negligible carbohydrate (Crombie, 1956) and a high rate of oxygen uptake would therefore be expected during the early stages of germination. The embryo is embedded in a mass of endosperm tissue which in turn is surrounded by the testa, and entry of water into these tissues was shown to be slow. It is well known that the diffusion of oxygen into compact tissues without intercellular spaces is restricted. It seemed likely therefore that in the kernel the oxygen supply to the embryo is below the level required for immediate germination. To test this, kernels were soaked and maintained in oxygen tensions higher than atmospheric at various temperatures. In a preliminary experiment which was run for a period of

2 months, kernels were maintained in air and in pure oxygen at temperatures of 25°, 33°, 40°, and 45° C. The results are presented in Table 2. During the 2 months, germination occurred only at 40° C. and the accelerating effect of oxygen is striking. The air controls at 40° C. reached a cumulative total of 5 per cent. after 8 weeks and this may be considered typical germination behaviour of fresh seed at high temperature. Table 3 shows the results of an experiment in which a wider range of temperatures and oxygen tensions was tested. The figures show the total percentage germination reached by each treatment after 8 weeks. The highest percentage occurred in pure oxygen at 40° C. It is clear from the results that the rate of germination increases with the oxygen tension but this effect is evident only at temperatures that fall within the optimum range for germination in air. High temperature appears to be indispensable for germination of the kernel but oxygen is clearly a limiting factor.

TABLE 2

The Effect of Oxygen on the Germination of tenera *Kernels at Various Temperatures*

Cumulative percentage germination

Temperature	Weeks 4	Weeks 8
25° C. Air	0	0
25° C. Pure oxygen	0	0
33° C. Air	0	0
33° C. Pure oxygen	0	0
40° C. Air	0	5
40° C. Pure oxygen	34	81
45° C. Air	0	0
45° C. Pure oxygen	0	0

TABLE 3

Influence of Temperature and Oxygen Concentration on the Germination of tenera *Kernels*

Cumulative percentage germination after 2 months

Oxygen concentration	30° C.	34° C.	36° C.	38° C.	40° C.	42° C.
20% (Air)	0	0	1	3	2	2
50%	0	0	4	36	37	7
75%	0	0	11	38	48	10
100% (Pure oxygen)	0	5	17	56	65	10

Table 4 shows the results of an experiment in which kernels were germinated in pure oxygen under pressure. One temperature only, 38° C., was tested. The first column shows the oxygen pressure expressed as a percentage of atmospheric pressure. The experiment was run for 4 weeks only. The results show that germination of the kernel may be accelerated still further by the use of oxygen under pressure. In 1·75 atmospheres of pure oxygen (representing approximately 8 times atmospheric partial pressure of oxygen) 60 per cent. germination was obtained, the 50 per cent. level being reached in approximately 3 weeks. Oxygen under a pressure of 2 atmospheres (200 per cent.) appeared to kill most of the seed. After 3 weeks, bacterial contamination of the seed in the high pressures of oxygen, viz. 150 and 175 per cent. was considerable, and most of the ungerminated seed appeared to be dead.

TABLE 4

The Effect of Pure Oxygen at Concentrations above Atmospheric Pressure on the Germination of tenera *Kernels at 38° C.*

Cumulative percentage germination

	Weeks	
Oxygen concentration	2	4
20% Air control	0	1
100% Pure oxygen (atmospheric pressure)	10	37
125%	5	23
150%	14	56
175%	27	60
200% (Twice atmospheric pressure) .	3	7

TABLE 5

The Effect of High Temperature and Oxygen on the Germination of tenera *Kernels*

Transfer experiment (1)

Cumulative percentage germination

First 4 weeks		Second 4 weeks	
Initial treatment	% germination	Transferred to	% germination
40° C. Oxygen	32	40° C. Oxygen (control)	78
		28° C. Air	67
		28° C. Oxygen	69
40° C. Air	0	40° C. Air (control)	5
		28° C. Air	9
		28° C. Oxygen	58
28° C. Oxygen	0	28° C. Oxygen (control)	1
		40° C. Air	3
		40° C. Oxygen	66
28° C. Air	0	28° C. Air (control)	0
		40° C. Air	0
		40° C. Oxygen	45

Transfer experiments. The experiments described above show that both high temperature and increased oxygen tension stimulate germination. In order to determine whether or not it is necessary to apply these treatments simultaneously, a transfer experiment was carried out. The first column in Table 5 specifies the initial treatments. Kernels were maintained in air and in pure oxygen at room temperature (which averaged 28° C.) and at 40° C., all for a period of 4 weeks. Germination after the first 4 weeks is shown in the second column. After this time, batches of 100 kernels were transferred to the alternative treatments shown in column three. The last column in the table shows the total percentage germination reached after the second period of 4 weeks (i.e. 4 weeks after transfer, or 8 weeks from the beginning of the experiment). Table 6 shows the results of a second experiment on the same lines which included extra treatments.

TABLE 6

The Effect of High Temperature and Oxygen on the Germination of tenera *Kernels*

Transfer experiment (2)

Cumulative percentage germination

First 4 weeks		Second 4 weeks	
Initial treatment	% germination	Transferred to	% germination
40° C. Oxygen	34	40° C. Oxygen (control)	75
		40° C. Air	40
		30° C. Air	49
		30° C. Oxygen	48
40° C. Air	2	40° C. Air (control)	12
		40° C. Oxygen	75
		30° C. Air	21
		30° C. Oxygen	41
30° C. Oxygen	1	30° C. Oxygen (control)	1
		30° C. Air	1
		40° C. Air	3
		40° C. Oxygen	12
30° C. Air	0	30° C. Air (control)	0
		30° C. Oxygen	0
		40° C. Air	0
		40° C. Oxygen	9

The results of both transfer experiments confirm the accelerating effect of oxygen and show that the response to increased oxygen tension is dependent upon an initial period of high temperature. It is seen that after an initial period at high temperature in air, germination is stimulated by transfer to oxygen at the lower temperature, but if the order of these treatments is reversed and the oxygen at low temperature given first, no such stimulation occurs on transfer to the high temperature in air. For oxygen to be effective therefore, high temperature must either precede or accompany the oxygen treatment, but given later it is ineffective. A definite sequence of reactions is suggested in which oxygen acts upon the products of the high temperature processes.

In the second transfer experiment shown in Table 6 the kernels germinating in air at 40° C. had reached a total of 12 per cent. after 2 months. Some 400 kernels in this treatment remained ungerminated and these were used for a further experiment, in which batches of 100 were transferred to the temperatures shown in the first column of Table 7. Half of each batch was placed in air, and the other half in pure oxygen. The third column in Table 7 shows the number of germinations that occurred in each batch during the 8 days following transfer and the last column the corresponding percentages. The treatments were continued for a period of 3 weeks following transfer, but in the 25°, 30°, and 35° C. treatments no further germination occurred after 8 days.

TABLE 7

The Effect of Temperature and Oxygen on the 'Flush' of Germination of tenera *Kernels induced after 2 months at 40° C. in Air*

(Germination at end of preliminary treatment (40° C. in air) 12%.)

Transferred to	Number of kernels transferred	Number of kernels germinated after 8 days in 'flush'	Percentage 'flush'
40° C. Air (control)	50	2	4
40° C. Oxygen	50	9	16
35° C. Air	50	4	7
35° C. Oxygen	50	11	20
30° C. Air	50	7	13
30° C. Oxygen	50	19	34
25° C. Air	50	11	20
25° C. Oxygen	50	8	14

Transfer to a lower temperature caused a 'flush' of germination and with the exception of 25° C. the 'flush' was greater in pure oxygen than in air at a given temperature. This phenomenon also occurred in the transfer experiments (Tables 5 and 6).

In Fig. 4 the 'flush' effect is shown graphically. In this graph certain of the results of the transfer experiments, Tables 6 and 7, are plotted. The curves originating from the lower dotted line (40° C. air controls) after 4 and 8 weeks, show the subsequent germination of samples transferred to alternative treatments. 'Flushes' of germination occurred when kernels were transferred to the lower temperature (30° C.); germinations were very frequent over a period of a few days, after which they quickly declined. When transferred from air to oxygen at the same temperature, 40° C., the frequency of germinations increased more slowly and continued over a longer period.

In a further experiment shown in Table 8, periods at high temperature in air were alternated daily with periods in pure oxygen at normal temperature (28–30° C.). These two treatments were alternated in time ratios of 1:9, 1:3, 1:1, 3:1, and 9:1 respectively as shown in the first column of Table 8. There were three control treatments: 40° C. in air, 40° C. in pure oxygen, and normal temperature (28–30° C.) in pure oxygen. The experiment was run for a period of 3 months.

In the control treatments, oxygen accelerated germination at 40° C. but was ineffective at the lower temperature. The results of the alternating treatments show that the longer the daily exposure to high temperature in air the faster was the germination and that even short periods of exposure to oxygen at the lower temperature stimulated germination. From these results, the relative rates of the processes occuring during the two treatments become clear. A daily exposure of the shortest period to oxygen at the lower temperature gives the maximal after-effect at high temperature in air, and it may therefore be concluded that the stage in the chain of processes dependent upon oxygen is rapid compared with the preceding reactions at high temperature. The transfer experiments described above have already shown that oxygen is only effective after a period at high temperature and that the oxygen must therefore act on a product of the high temperature processes.

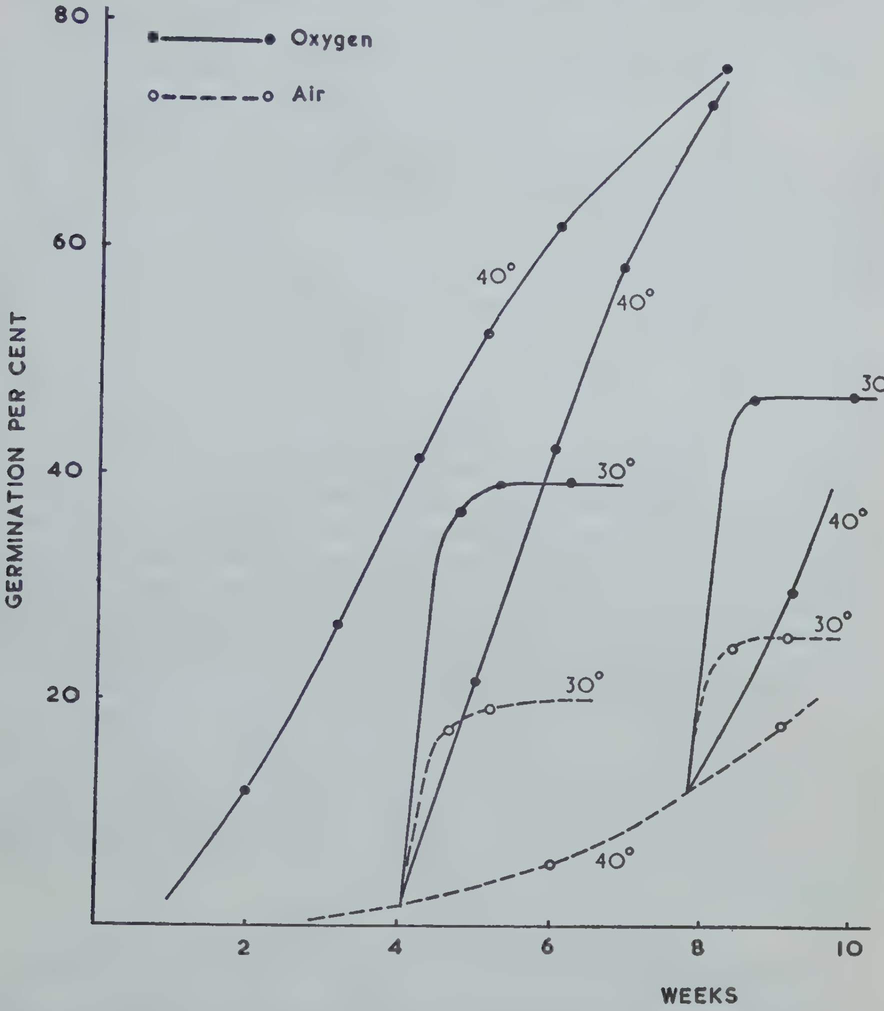


Fig. 4. Germination curves for various treatments shown in Tables 6 and 7. The curves originating from the lower broken line (40° C. air controls) after 4 and 8 weeks shown subsequent germination of samples transferred to alternative treatments.

TABLE 8

The Effect of Alternating Temperatures on the Germination of tenera *Kernels*

40° C. in air alternated with 30° C. in oxygen in time ratios of 9:1, 3:1, 1:1, 1:3, 1:9 with the length of the cycle constant at 24 hours

Cumulative percentage germination

Ratio: 28–30° C. Oxygen:40° C. Air (24-hour cycle)	Months 1	2	3
1:9 (2·4 hours:21·6 hours)	1	23	70
1:3 (6 ,, :18 ,,)	3	16	49
1:1 (12 ,, :12 ,,)	5	12	14
3:1 (18 ,, : 6 ,,)	5	8	9
9:1 (21·6 ,, : 2·4 ,,)	1	4	5
Control treatments			
40° C. Air	0	7	30
40° C. Oxygen	40	75	90
28–30° C. Oxygen	0	0	0

Optimum temperature for germination of kernels. The results of the experiment shown in Table 3 indicate the optimum temperature for germination, viz. 38–40° C., in the higher oxygen tensions only. Owing to the restricted duration of the experiment, only a few germinations had occurred in the air controls and there is no evidence from the results for the optimum temperature for germination in air. At the close of the experiment, however, the air controls were left on the laboratory bench at a temperature of 25–27° C. Table 9 shows the 'flush' of germination that occurred following each temperature treatment. If the percentage 'flush' can be taken as a measure of the progress of the high-temperature effect during the preceeding 2 months, then a clear optimum of 40° C. is indicated. A temperature of 42° C. appeared to be lethal. The figures show that the optimum range for the high temperature process is narrow, viz. 38–40° C., and a sharp critical temperature is suggested.

TABLE 9

Influence of Temperature and Oxygen Concentration on the Germination of tenera *Kernels*

Percentage 'flush' of germination after 3 weeks in air at 25–27° C. following transfer after 2 months in air at temperatures ranging from 30° C. to 42° C.

30° C.	34° C.	36° C.	38° C.	40° C.	42° C.
0	2	11	53	70	0

EXPERIMENTS WITH DISSECTED KERNELS

The experiments with excised embryos showed that when isolated and placed on moist filter paper, the embryo germinates within 24 hours at temperatures of 28–30° C. Kernels have been kept fully imbibed at these temperatures, both in air and in pure oxygen for periods of up to 6 months without any sign of germination. The tissues that normally surround the embryo, viz. endosperm and testa, must inhibit the growth of the embryo in some way. There are three main possibilities; firstly, the surrounding tissues may restrict the diffusion of oxygen to the embryo, secondly, they may mechanically obstruct the extension growth of the embryo, and thirdly, they may contain a germination inhibitor.

The experiments described in the previous section demonstrated at least two factors concerned in the dormancy of the kernel. One factor is clearly lack of oxygen, the other unknown factor is temperature dependent. No facilities were available for testing the growth activity of tissue extracts, and in order to gain further information on the nature of the inhibiting factor present, some simple experiments were carried out in which different portions of the kernel in the region of the embryo were dissected away. These tests were carried out with freshly soaked kernels under sterile conditions. The kernels were then maintained in a saturated atmosphere for a period of 4 days at 30° C. after which any elongation of the embryo was measured. Replication in these tests was 20 kernels per treatment. The extension growth of embryos in dissected kernels was compared with that of embryos excised from the same batch of kernels, germinated on moist filter paper at 30° C. The details of the various treatments, together with the results, are illustrated diagrammatically in Fig. 5. Diagram A represents an embryo *in situ* surrounded by the endosperm and testa; at 30° C. (either in air or in pure oxygen) no germination occurred after a period of 6 months. The excised embryos, B, elongated $0{\cdot}4 \pm 0{\cdot}13$ mm. in 4 days (original length approximately 3 mm.).

De-operculation. By careful microdissection, the circular disc of endosperm and testa that forms the operculum may be removed without damage to the embryo, Fig. 5 c. When the operculum has been thus removed the distal surface of the embryo is exposed to atmospheric oxygen and the embryo is free to elongate. The embryo in such kernels emerged very slowly with a mean rate of elongation of $0{\cdot}21 \pm 0{\cdot}1$ mm. in 4 days, half the rate of elongation of excised embryos over the same period. The individual rates of elongation were very variable and in about 20 per cent. of the replicates, the response was barely perceptible. Most of the kernels became contaminated before any appreciable elongation had occurred.

The above test was repeated using two batches of 20 kernels; one batch was maintained in air at 30° C. (as before), the other in pure oxygen at the same temperature. The results of the test are given in Table 10. In air the embryo emergence was again very slow and the kernels behaved as before; in oxygen, however, the emergence was much faster and the embryos continued to elongate after the first 4 days like embryos emerging from germinated whole kernels; most of these produced normal seedlings. Plate 2, Fig. 4 shows three kernels that have been split longitudinally in order to show the embryos clearly. The operculum has just been removed from the kernel on the left. The remaining two de-operculated kernels have been maintained in air and in pure oxygen respectively for 4 days at 30° C.

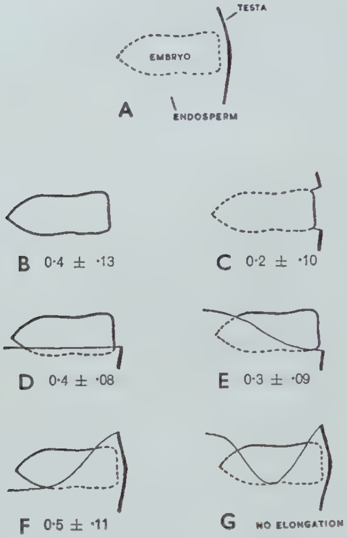


FIG. 5. Diagrams illustrating the various treatments in the experiments with dissected kernels. For explanation see text. Figures give mean elongation of embryos after 4 days at 30° C. Original length of embryo approximately 3 mm. (The thickness of the layer of endosperm covering the distal part of the embryo has been slightly exaggerated; compare Plate 2, Figs. 4, 5, and 6.)

TABLE 10

The Effect of Oxygen on the Germination of Fresh De-operculated Kernels at 30° C.

Original length of embryo 3 mm.

Emergence of embryo in millimetres after 4 days at 30° C.

Whole kernels	nil
Decapped kernels in air	0·23±0·1
Decapped kernels in oxygen	1·02±0·2

Split kernels. By splitting kernels longitudinally, the embryo may be exposed on one side only while its opposite side remains in contact with the endosperm. Sets of kernels were split so that the embryos were exposed in the various ways illustrated diagrammatically in Fig. 5 D, E, F, and G. When only a small part of the embryo was exposed as in treatment G, while both the haustorium and the distal end remained buried, no elongation took place. Any elongation would have ruptured the operculum. When the embryo was exposed so that either end or both ends were free, as in treatments D, E, and F, extension growth took place. The increments in length after the first 4 days are shown in Fig. 5; none differed significantly from the increments for excised embryos. After the first 4 days most of the embryos in D, E, and F elongated considerably, with extension growth increments of up to 5 mm. in 8 days. Elongation after the first 4 days, however, was erratic and about half the split kernels became contaminated.

The results of these experiments may be summarized as follows: (1) It is not necessary to separate the embryo from the endosperm in order to obtain immediate germination. Contact with the endosperm does not appear to have any inhibiting effect on the embryo. (2) The partly exposed embryo will germinate immediately only if it is free to elongate. The failure of the embryo in treatment G to show any elongation suggests that the operculum offers some mechanical resistance to the embryo. (3) When the operculum is removed, treatment with high temperature is no longer necessary, germination is controlled only by the oxygen tension. At 30° C. in air or in pure oxygen kernels remain ungerminated for at least 6 months. At the same temperature, with the operculum removed, germination begins within a few days, the rate of embryo emergence increasing with the oxygen tension.

Germination of embryos from kernels imbibed for 2 months at normal and high temperatures. The experiments with whole kernels show that at 40° C. in air there is an initial lag period of 6–8 weeks during which a small percentage only (5–10 per cent.) of the seed germinates. If after this time the temperature is lowered to 25–30° C. a 'flush' of germination takes place, when up to 50 per cent. may germinate within a few days, according to the oxygen tension. It was found that if, in addition to lowering the temperature, the operculum is removed from such kernels, 100 per cent. germination occurs within 24 hours. The rate of embryo emergence from these de-operculated kernels in air at 30° C. was much faster than the rate for de-operculated fresh kernels. To examine this effect more critically, the experiment shown in Table 11 was carried out. The second column in this table shows the results of a series of tests carried out with fresh kernels. The extension growth of excised embryos at 30° C. was compared with the rate of emergence of embryos in de-operculated kernels at the same temperature (see also Tables 1 and 10). The remaining kernels were then maintained in air in a fully imbibed condition for a period of 2 months at temperatures of 30° and 40° C. After this time a similar series of tests was carried out on kernels from each of the two temperature treatments. The results of these tests are shown in columns three and four of Table 11. After the 2 months' treatment, 2 per cent. only of the kernels had germinated at 40° C. while none had germinated at the lower temperature. When transferred to 30° C. in pure oxygen, a sample of kernels from the high temperature treatment gave a 'flush' of germination of 50 per cent. in 4 days.

The results show: (1) That embryos excised from seed treated for 2 months germinated more rapidly than those excised from fresh seed, but that the temperature during the 2 months pre-treatment (i.e. either 30° or 40° C.) did not affect the subsequent rate of extension growth. (2) Embryos germinating in the de-operculated kernels show the reverse of this. The rate of embryo emergence was affected by the pre-treatment only at the higher temperature of 40° C. This experiment was repeated and similar results obtained. The implications of these effects will be considered in the discussion below.

TABLE 11

Germination of Excised Embryos and De-operculated Kernels after Pre-treatment of Seed (kernels) for 2 months at 30° C. and 40° C.

	Replication	Fresh kernels (control)	Preliminary treatment for 2 months	
			30° C. in air	40° C. in air
Excised embryos				
Dry weight mgs.	40	1·41	1·42	1·47
Initial length mm.	25	3·60±0·6	3·61±0·5	3·86±0·6
Increment in length after 4 days at 30° C.	25	0·33±0·15	1·34±0·14	1·34±0·17
Increment in length after 8 days at 30° C.	25	1·07±0·30	2·17±0·28	1·95±0·27
De-operculated kernels				
Emergence in mm. after 4 days at 30° C.	40	0·30±0·10	0·30±0·11	1·30±0·21
Percentage 'flush' germination of whole kernels after 4 days at 30° C. in pure oxygen	100	—	0	50%

EXPERIMENTS WITH GROWTH SUBSTANCES

The effect of IAA, 2,4-D, and ethylene chlorhydrin on the germination of whole kernels, de-operculated kernels, and excised embryos

The following tests were carried out to determine any possible effects of indoleacetic acid, 2,4-dichlorophenoxyacetic acid, and ethylene chlorhydrin on the germination of kernels, dissected kernels, and excised embryos.

Fresh *tenera* kernels maintained in air at temperatures of 30° and 40° C. were immersed daily for a period of 1 hour in freshly prepared sterile aqueous solutions of IAA and 2,4-D at concentrations of 100 p.p.m., 10 p.p.m., and 1·0 p.p.m., and of ethylene chlorhydrin at concentrations of 1·0 and 0·1 p.p.m. Controls were soaked in sterile tap water. The soaking treatments were continued for 2 weeks. No germination occurred during the following 4 weeks in any of the treatments, after which the experiment had to be discontinued. Kernels from the same batch gave 40 per cent. germination in pure oxygen at 40° C. after 6 weeks.

De-operculated kernels were soaked for 24 hours in sterile aqueous solutions of IAA at concentrations of 100, 10, and 1·0 p.p.m. The rate of embryo emergence in air after 4 days at 30° C. was similar to that in de-operculated kernels soaked in sterile tap water (0·2–0·3 mm.). Excised embryos were floated on solutions of IAA at concentrations of 100, 10, and 1·0 p.p.m. at

30° C. These embryos elongated at the same rate as embryos floated on sterile tap water, approx. 0·4 mm. in 4 days.

No inhibiting or promoting activity of the substances tested was evident.

DISCUSSION

The experiments with whole nuts will be described fully elsewhere and a brief summary only is given at the end of this paper. The germination behaviour of the kernel is modified by the presence of the shell only at high moisture levels when the endocarp tissues appear to restrict still further the entry of oxygen. The central problem of dormancy in the oil-palm seed concerns mechanisms present in the kernel; these will now be considered.

When excised from a fresh seed and placed on moist filter paper, the embryo displays growth activity within 24 hours and may therefore be considered non-dormant. Although the initial growth-rate increases with temperature, especially over the range of 25–30° C., high temperatures of 35–40° C. are not necessary for elongation of the isolated embryo. The initial rate of extension growth of the excised embryo is very slow compared with the initial rate of emergence of the embryo germinating *in situ*. This can be seen in Fig. 6 where increments in embryo length are plotted against time. The curves for embryos emerging from whole kernels (A, B, and C) were obtained from mean values of measurements made on the kernels germinating in 'flushes' at 25°, 30°, and 35° C. respectively (see Table 7). The points of origin of these curves on the abscissa show the mean number of days to germination of each flush (air and oxygen treatments combined) from the time of transfer from the high temperature (40° C.). Curves D and E show the rates of extension growth of excised embryos germinating on moist filter paper at 30° C., D represents embryos excised from kernels kept for 8 weeks at 40° C. in air (see Table 11) and E represents embryos excised from fresh seed.

It would be expected that the embryo would germinate more slowly in the absence of nutrients normally supplied by the endosperm, but even when half the surface of the embryo was in contact either with endosperm tissue or with sucrose agar, the rate of extension growth over the first 4 days did not differ from that of the excised embryo on moist filter paper. After the first 4 days the extension growth of the excised embryo on filter paper gradually diminishes until it finally ceases after approximately 6–8 days. On sucrose agar, growth continues after 8 days but becomes progressively more sluggish. When in contact with the endosperm, however, the growth-rate increases considerably after 4 days as was shown in treatments D, E, and F of the experiment shown in Fig. 5. Growth of the embryo thus appears to be dependent upon the endosperm but in the absence of proper facilities for tissue culture this could not be studied further. Growth factors similar to those found in the coco-nut endosperm may be involved.

In the ungerminated kernel the operculum is demarcated by a circular abscission layer. Plate 2, Fig. 8 shows that fracture in the abscission zone takes place along the middle lamellae between endosperm cells that lie between the testa and the distal end of the embryo. Where these cells meet the embryo the walls are thickened so as to form a ring of strengthening tissue at the base of the operculum. Within this structure is a circular groove which appears as a small cavity in longitudinal section. This groove determines the direction of fracture and may also serve as a channel for pectic enzymes secreted by the embryo.

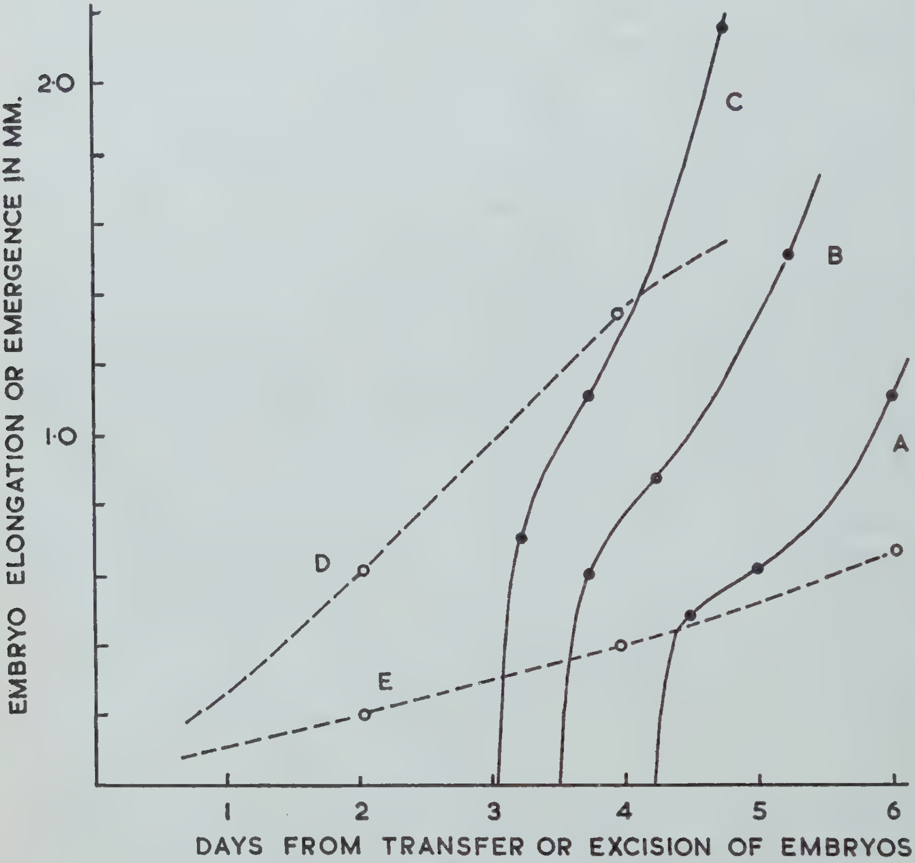


Fig. 6. Comparison of the rates of emergence of embryos from germinating intact kernels (curves A, B, and C) with the rates of elongation of excised embryos (curves D and E). For explanation see text of Discussion.

The evidence suggests that the functioning of the abscission layer is dependent upon the cessation of dormancy in the embryo, and that until the middle lamellae in the abscission cells have been effectively broken down, the operculum forms a mechanical barrier and prevents the embryo from emerging. The failure of the partly exposed embryo to elongate when neither end is free (treatment G Fig. 5) suggests that the operculum may temporarily obstruct extension growth of the embryo. Curves A, B, and C in Fig. 6 show that the embryo emerges rapidly from an intact kernel during the few hours following rupture of the operculum. After this the rate of emergence slows down before gradually increasing again. The curves are altogether different from those for the excised embryo. This initial rapid emergence of the embryo is consistent with a sudden release of tension after pressure has been applied to a structure that abruptly gives way. When excised, the embryo is free to elongate and cell division and cell elongation may proceed simultaneously. While embedded in the endosperm, however, extension growth cannot take place until the operculum is ruptured; there is no room for the fully imbibed embryo to expand *in situ*. The existence of a lag period between the initiation of growth activity in the embryo and the functioning of the abscission layer would mean that cell division in the embryo would not be followed by cell extension until the operculum had been ruptured. Once rupture had taken place, a wave of cell extension would follow and cause rapid emergence of the embryo. The rate of emergence would slow down before gradually increasing again as the rates of cell extension and cell division became readjusted. Such a mechanism would account for the differences in the curves shown in Fig. 6. A lag period before rupture of the operculum of 3–4 days according to the temperature would be involved.

Wellington (1956) has shown that the delay in germination in the red variety of wheat grains may be attributed to the constraint imposed by the pericarp. Emergence of the embryo appears to be delayed until there has been an increase in the water-absorbing power of the embryo, thus creating sufficient turgor pressure to rupture the pericarp. In the oil-palm seed the rupture of the operculum appears to be dependent upon intercellular breakdown in the abscission layer as well as on the growth pressure of the embryo. The functioning of the abscission layer may in turn be dependent upon pectic enzymes secreted by the embryo.

The dormancy of the intact kernel is controlled by the temperature and the external oxygen tension. At 25–30° C., in normal atmospheric oxygen tensions, the dormant period may be a matter of years, but with increasing temperature up to a maximum of 40° C. the delay in germination is progressively reduced to a few months. The dormant period is shorter when kernels are germinated in pure oxygen (see Table 3) but it was possible to detect the accelerating effect of oxygen only at fairly high temperatures. Oxygen may still accelerate germination at 25–30° C. but it would be necessary to continue the experiment over a period of years in order to test this.

The transfer experiments with whole kernels (Tables 5, 6, and 7) show that the accelerating effect of oxygen is a secondary effect dependent upon the progress of a slow reaction with a high optimum temperature. After a long period at high temperature, a 'flush' of germination is initiated if the temperature is lowered. The 'flush' is greater in pure oxygen than in air. Kernels that remain ungerminated after the 'flush' has occurred presumably require more treatment at high temperature in order to carry the reaction to the stage where an increase in the oxygen tension is effective. James (1953) points out that the oxygen concentration in plant tissues rapidly diminishes with increasing temperature above 25° C., an effect which he attributes to the high temperature co-efficient of respiration and the reduced solubility of oxygen at higher temperatures. Burton (1950) has shown that in potato tubers the oxygen saturation falls from 49 per cent. at 28° C. to 10 per cent. at 37° C. It would be expected therefore that lowering the temperature from 40° C. to 30° C. would alone increase the availability of oxygen within the embryo tissues and this would undoubtedly accelerate germination. The stimulating effect on germination of short periods (12–48 hours) at lower temperature, interrupting a treatment at 38° C., noted by Henry (1951) would appear to involve the same mechanism as the 'flush' effect described here.

Once the high-temperature requirement has been satisfied in the intact kernel, germination appears to be controlled only by the oxygen tension, a situation similar to that in the de-operculated kernel that has received no high-temperature treatment. If the operculum is removed from a fresh kernel, growth activity of the embryo is evident within a few days at 30° C. but the rate of emergence is very slow unless the de-operculated kernel is placed in pure oxygen. In the higher oxygen tension germination proceeds normally at 30° C. and seedlings may be obtained. Intact kernels under identical conditions remain ungerminated for at least 6 months. The presence of the operculum will of course reduce the availability of oxygen within the embryo but it is difficult to account for this striking difference in behaviour by oxygen deficiency alone, unless some mechanism involving a 'threshold' concentration of oxygen within the embryo tissues is postulated.

The results of the experiment shown in Table 11 may be interpreted in two ways. High temperature during the preliminary 8 weeks may accelerate changes in the endosperm, or alternatively the embryo may be modified at high temperature so that it subsequently makes more efficient use of the endosperm. Reference to Tables 10 and 11 shows that the rate of embryo emergence in air following de-operculation is faster after 8 weeks at 40° C. and equivalent to the rate of emergence of an embryo in a de-operculated fresh kernel placed in pure oxygen. The oxygen concentration required for a given rate of embryo emergence has thus been reduced by 8 weeks' treatment at 40° C., but not at 30° C.

The data presented suggest that oxygen-supply is the principal factor involved in the dormancy mechanism. It would appear that germination is dependent upon a minimal concentration of oxygen within the embryo tissues. In the fresh kernel this minimum is not reached unless the surrounding tissues or part of them are removed. Increasing the external oxygen tension to 100 per cent. is not sufficient to initiate germination of the intact kernel at 30° C. (oxygen pressures higher than atmospheric were not tested at 30° C.). During dormancy the minimum oxygen concentration required by the embryo to start growth gradually decreases at a rate depending on the temperature. There may be several possible factors contributing to this effect such as a general increase in oxidizable material or the destruction of an oxidizable inhibitor or its precursor. Acceleration of this process would appear to be the main effect of high temperature on germination.

The low permeability of the tissues surrounding the embryo, as well as restricting the inward diffusion of oxygen, may also cause accumulation of CO_2 within the embryo tissues. It is possible that high concentrations of CO_2 may be inhibitory and play some part in the dormancy mechanism.

Germination of the intact kernel is marked by rupture of the operculum. The evidence presented above, however, suggests that growth activity actually begins in the embryo some days before rupture which is delayed until the middle lamellae between the cells of the abscission layer are weakened, presumably by pectic enzyme activity.

Of the three possible factors controlling germination suggested on page 273 the effects of oxygen and mechanical constraint have now been discussed. There remains the question of the presence of an inhibitor. Evenari (1949, 1957) has drawn attention to the wide occurrence of germination inhibitors and recently Wareing and Foda (1957), using chromatographic methods, have demonstrated the presence of two inhibiting substances in the seed of *Xanthium pennsylvanicum*. In this species it is well known that oxygen plays an important role in controlling germination (Crocker, 1948). From the work of Wareing and Foda it appears that germination of the *Xanthium* embryo will not take place until the level of inhibitors has been effectively reduced by oxidative reactions. They point out that a similar situation exists in certain varieties of lettuce in which dormancy may be broken either by removing the seed coat (Evenari and Neumann, 1952) or by exposure to increased oxygen tensions (Borthwick and Robbins, 1928); these seeds appear to contain two inhibitors, which, according to Wareing and Foda, have properties similar to those found in *Xanthium*. The excised upper embryo of *Xanthium* will not germinate in normal atmospheric oxygen tensions but will do so in pure oxygen. In showing this, Wareing and Foda germinated the embryos on glass in 100 per cent. humidity, in order to prevent leaching of the inhibitor which occurs during soaking of the naked embryos but not during soaking of the intact seed. In earlier work, Thornton (1935) found the minimal concentration of oxygen required for germination of the upper excised embryo was approximately 1 per cent.; Wareing and Foda attribute this lower value to leaching caused by contact with moist cotton on which the embryos were placed. In the work described here with oil-palm embryos, leaching could have occurred in embryos germinated on moist filter paper but not in the partly exposed embryos germinating in split kernels, which were not exposed to free water but kept in a saturated atmosphere. The fact that exposure of part of the embryo to water-saturated air while still in contact with the endosperm leads to immediate germination, argues against the presence of an inhibitor, or alternatively that rapid oxidation of any inhibitor takes place in air. The experiment shown diagrammatically in Fig. 5 cannot alone decide this issue.

The results of this investigation do no more than demonstrate the main factors affecting germination of the oil-palm seed and suggest the type of mechanisms involved. Some of the phenomena reported here have been shown to occur in a number of other seeds, viz. wheat, lettuce, and *Xanthium*; oil-palm seed may thus prove to be useful material for further studies in germination.

EXPERIMENTS WITH OIL-PALM NUTS

To investigate the effect of moisture on the germination of oil-palm nuts, the latter were kept at different moisture levels for long periods, both in air and in pure oxygen. The results show that with increasing moisture content above the minimum required to imbibe the embryo, germination is progressively delayed. Nuts fully saturated with water fail to germinate even in pure oxygen at 40° C. At high moisture contents, the permeability of the endocarp to oxygen is considerably reduced by water films blocking the intercellular spaces. To obtain the maximum rate of germination in practice, it is therefore necessary to control the moisture content of the nuts as well as the temperature.

SUMMARY

1. Oil-palm seedlings are germinated from 'nuts' which consist of the endocarp, or shell, enclosing one, two, or occasionally three kernels. The kernel comprises a small embryo embedded in a mass of oily endosperm which in turn is completely surrounded by a thick testa.

2. The distal part of the embryo is separated from the testa by a thin layer of endosperm which contains a circular abscission layer. When separated kernels are allowed to germinate, the endosperm within the abscission layer together with the adjacent portion of the testa are forced out of the kernel by the elongating embryo. The disc of tissue forced out constitutes an operculum.

3. Ground temperatures in West Africa where oil palms are grown average 25–30° C., and within this range both nuts and kernels germinate sporadically over a period of years. With increasing temperature, however, the long dormant period is progressively reduced; at 38–40° C. 50 per cent. germination may be obtained after a few months. Temperatures in excess of 40° C. are lethal.

4. Kernels germinate faster in increased oxygen tensions but oxygen is effective only at high temperatures of 35–40° C., or at lower temperatures after the seed has been maintained at high temperature in air for a long period. The accelerating effect of oxygen is shown to be dependent upon the progress of slow processes stimulated by high temperature. At a constant temperature of 30° C., imbibed kernels remain ungerminated for at least 6 months either in air or in pure oxygen. After a preliminary period of

2 months in air at 40° C., up to 50 per cent. germination may occur within a few days on transfer to pure oxygen at 30° C.

5. At 30° C. the excised embryo begins to elongate within 24 hours on moist filter paper. The initial rate of elongation is not affected by contact with endosperm tissue or with sucrose agar. After the first 4 days the growth of the excised embryo is dependent upon nutrient factors in the endosperm in addition to salts and sugar.

6. Removal of the operculum is followed by sluggish emergence of the embryo at 30° C. in air. At the same temperature in pure oxygen, however, the embryo emerges more rapidly and normal seedlings are produced. After preliminary treatment with high temperature (40° C. in air for 2 months) de-operculation is also followed by more rapid emergence of the embryo in air at 30° C.

7. The germination of kernels, de-operculated kernels, and excised embryos is not affected by IAA or 2,4-D. Ethylene chlorhydrin is without effect on the germination of kernels.

8. It is suggested that germination of the oil-palm seed is dependent upon a minimal threshold concentration of oxygen within the embryo tissues. During dormancy this minimal level progressively decreases at a rate depending on the temperature. The evidence for an inhibitor is discussed.

ACKNOWLEDGEMENTS

This work was carried out during secondment to the West African Institute for Oil Palm Research, Nigeria, and my thanks are due to the former Director Mr. F. W. Toovey for his advice and encouragement. I am especially grateful to Professor F. G. Gregory, F.R.S. for much useful discussion in the preparation of this paper.

LITERATURE CITED

BEIRNAERT, A., 1936: Germination of the Seed of *Elaeis*. Publ. de l'I.N.E.A.C. Ser. Tech., No. 4.

BORTHWICK, H. A., and ROBBINS, W. W., 1928: Lettuce Seed and its Germination. Hilgardia, **3**, 275.

BUNTING, B., EATON, B. J., and GEORGI, C. D. V., 1927: The Oil Palm in Malaya. Malayan agric. J., **15**, 299–386.

—— GEORGI, C. D. V., and MILSUM, J. N., 1934: The Oil Palm in Malaya. Kuala Lumpur Dept. Agric.

BURTON, W. G., 1950: Studies on the Dormancy and Sprouting of Potatoes 1. The Oxygen content of the Potato tuber. New Phyt., **49**, 121–34.

CROCKER, W., 1948: Growth of Plants. Reinhold Publishing Corporation, New York.

CROMBIE, W. M., 1956: Fat Metabolism in the West African Oil Palm (*Elaeis guineensis*) Part 1. Fatty Acid Formation in the Maturing Kernel. Jour. exp. Bot., **7**, 181–93.

EVENARI, M., 1949: Germination inhibitors. Bot. Rev. **15**, 153.

—— 1957: Symposia of the Society for Experimental Biology. No. 11. The Biological Action of Growth Substances, 21–43.

—— and NEUMANN, G., 1952: The Germination of Lettuce Seed 2. The influence of the fruit coat, seed coat, and endosperm upon germination. Bull. Res. Council Israel, **2**, 75.

FERWERDA, J. D., 1956: Germination of Oil Palm Seeds. J. Imperial Col. trop. Agric. **33**, 51–66.

GALT, R., 1952: W.A.I.F.O.R. Report (unpublished).
HENRY, P., 1951: La Germination des graines d'Elaeis. Rev. Bot. appl., **31**, 349.
JAMES, W. O., 1953: Plant Respiration. Clarendon Press, Oxford.
MILSUM, J. N., 1927: Hastening the Germination of Oil Palm Seeds. Malay agric. J., **15**, 82.
RUTGERS, A. A., 1922: Investigations on Oil Palms (edited by A. V. R. O. S. Medan). Batavia, 1922.
THORNTON, N. C., 1935: Factors Influencing Germination and Development of Dormancy in Cocklebur Seeds. Cont. Boyce Thompson Inst., **7**, 477.
TOOVEY, F. W., 1937: Unpublished Report.
VANDERWEYEN, R., 1952: Notions de culture de l'Elaeis au Congo Belge. Brussels.
WAREING, P. F., and FODA, H. A., 1957: Growth Inhibitors and Dormancy in *Xanthium* Seed. Phys. Plant., **10**, 266–80.
WELLINGTON, P. S., 1956: Studies on the Germination of Cereals. 2. Factors Determining the Germination Behaviour of Wheat Grains during Maturation. Ann. Bot., N.S., Lond., **20**, 481–500.
YAMPOLSKY, C., 1922: A Contribution to the Study of the Oil Palm. Bull. Jard. Bot. Buitenzorg, **3**, 107–74.

EXPLANATION OF PLATES

FIG. 1. Excised embryos. Top: freshly excised; middle: after 8 days on moist filter paper at 40° C.; bottom: after 12 days on sucrose agar at 30° C. (×5)

FIG. 2. Excised embryos after 25 days on sucrose agar at 30° C. The haustorium has not developed and has darkened. The embryo on the left shows development of the primary root only, the embryo on the right has developed the shoot only. (×5)

FIG. 3. Ungerminated *tenera* nut split longitudinally showing the embryo *in situ*. See Text-fig. 1. (×12)

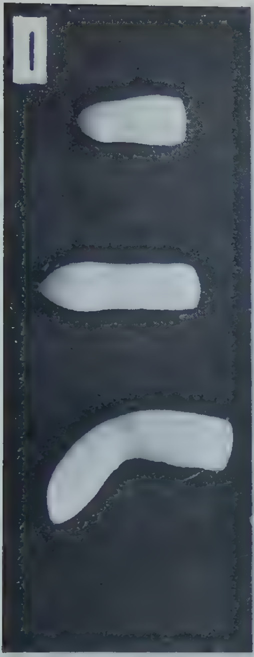
FIG. 4. De-operculated kernels split to show the embryo. Left: freshly de-operculated, middle: after 4 days in air at 30° C.; right: after 4 days in pure oxygen at 30° C. (×3)

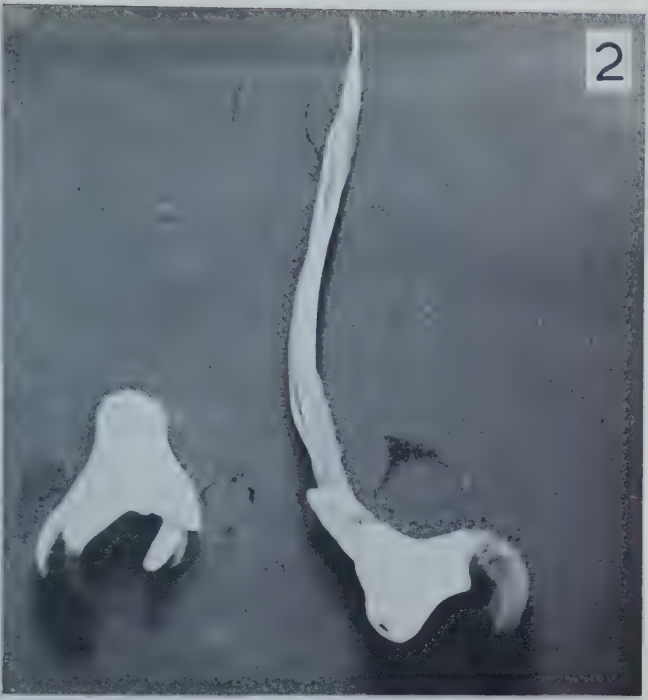
FIG. 5. Just-germinated kernel showing the operculum being forced out by the elongating embryo. (×5)

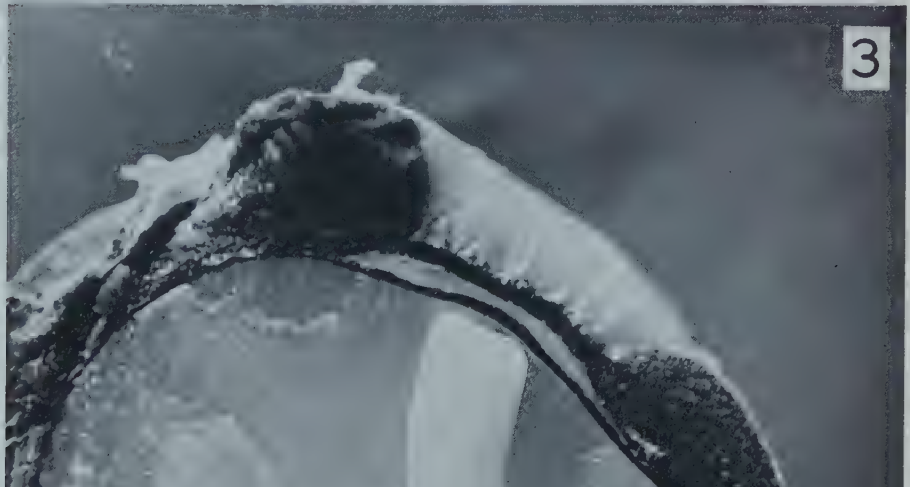
FIG. 6. Germinated kernel showing operculum still adhering to the distal part of the emerging embryo. (×5)

FIG. 7. L.S. ungerminated *tenera* kernel showing the distal part of the embryo (bottom left) covered by the testa (dark layer on right) and thin layer of endosperm. The periphery of the operculum is demarcated by the abscission zone seen in the centre. (×60)

FIG. 8. L.S. of ungerminated *tenera* kernel which has received heat treatment (40° C. in air) for 2 months and fixed 2 days after transfer to pure oxygen at 30° C. The drag of the microtome knife has been sufficient to rupture the abscission layer where the cells have parted along the middle lamellae. (×550)

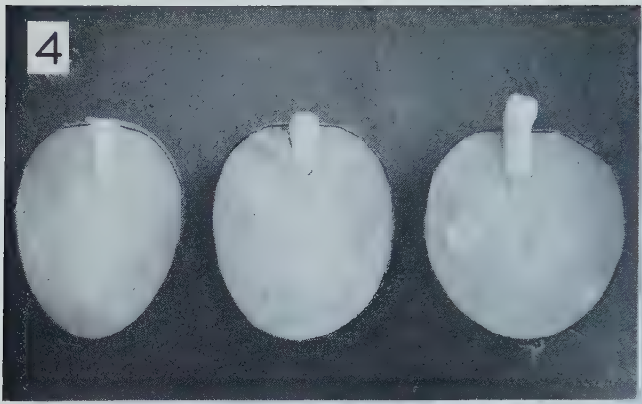




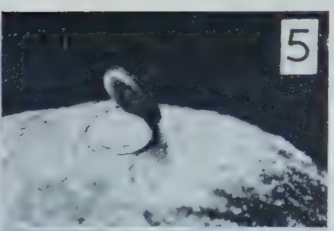


G. HUSSEY

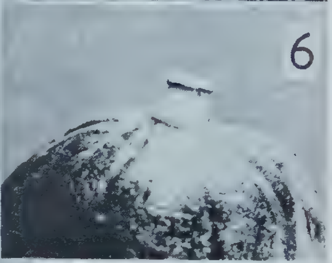
4



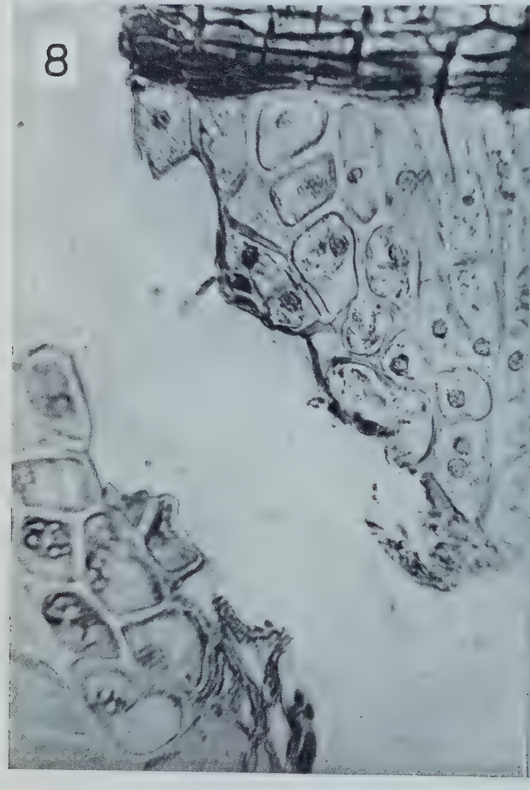
5



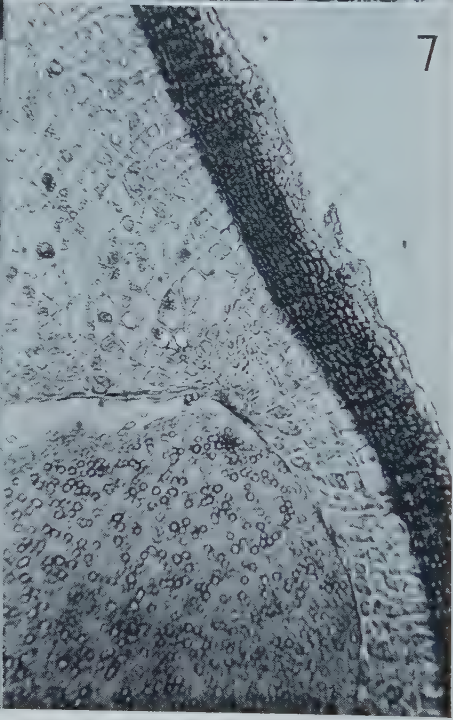
6



8



7



G. HUSSEY

The Effect of Wind Speed on Assimilation Rate—a Re-assessment

BY

J. WARREN WILSON AND R. M. WADSWORTH

(*Botany Department, University of Reading*)

With two Figures in the Text

ABSTRACT

Deneke concluded from wind-tunnel experiments that CO_2 uptake is increased by wind, but that this stimulation is proportionately less at higher wind speeds, and that above 100 m./min. (167 cm./sec.) no further increase results. His data in fact indicate a linear relationship between wind and assimilation rate over the whole range investigated. The wind speeds he used were of the same order as those occurring in vegetation in the field.

Published experiments on the effect of wind on dry-weight increase, on the other hand, show that wind reduces growth; but these experiments have used winds equivalent to 'gale' or 'hurricane', and at such speeds other, harmful effects of wind become important.

Wind-tunnel investigations on stands of *Brassica napus* reconcile these observations by showing growth-rates to increase with wind speed up to an optimum near 30 cm./sec., and to fall off at higher speeds. It is considered that the stimulating effect of wind on assimilation shown by Deneke's and these experiments is likely to be significant in the field.

THE only investigation that has been made of the effect of wind speed on carbon dioxide (CO_2) uptake by plants seems to be that of Deneke (1931). It had previously been known—and Deneke's experiments confirmed it—that if the flow of air past a leaf is restricted, as in certain techniques for measuring photosynthesis, the assimilation rate falls as a result of the reduction in the CO_2 content of the air (e.g. Kostytschew, Bazyrina, and Wassilieff, 1927). Deneke investigated this relationship quantitatively; his conclusion was that the acceleration of CO_2 uptake resulting from increase in wind speed becomes proportionately smaller as the wind speed rises, and that above 100 m./min. (167 cm./sec.) increase in wind speed produces no further increase in CO_2 uptake. 'Die Beeinflussung durch die Windgeschwindigkeit ist bei geringer Luftbewegung ziemlich groß. Bei Erhöhung der Windgeschwindigkeit wird der Effekt prozentual kleiner, bis bei ungefähr 100 m./min. eine weitere Erhöhung der Windgeschwindigkeit keine Erhöhung der Kohlendioxydaufnahme mehr mit sich bringt.'

This conclusion is not supported by a re-examination of his data. On the contrary these show, when suitably expressed, that CO_2 uptake was accelerated by increase in wind speed at the highest velocities examined, just as at low speeds. The rate of CO_2 uptake was linearly related to wind speed over the

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

whole range. To explain this re interpretation of Deneke's results it is necessary to outline his methods.

Deneke used an air-tight, 'closed circuit', wind tunnel enclosing 20 litres of air which was continuously circulated by a fan. The experimental plants were placed in an artificially illuminated 'working section' which was 4 cm. by 6 cm. across; here, wind speeds of up to 56 m./min. (93 cm./sec.) could be obtained. The rate of CO_2 uptake was gauged by measuring the time taken for the CO_2 concentration of the air in the tunnel to fall by a standard amount, estimated colorimetrically; the higher the rate of uptake the shorter was the time interval (there being a reciprocal relationship between these two values). Several species with different stomatal arrangements were examined. Wherever possible whole potted plants were used; but in two cases out of six, cut shoots had to be employed (*Thuja gigantea* and *Nerium oleander*). Each observation was replicated and agreement was good.

All six species gave results of the same type; those for *Nerium* can be taken as an example, since Deneke treats these most fully. He presents his results in the form shown in Fig. 1(*a*), with wind speed plotted against time taken for a standard amount of CO_2 uptake. His extrapolation suggests that at higher wind speeds the curve becomes parallel to the wind speed axis. The curves for the other species can be extrapolated similarly. These graphs are the basis for his conclusion that increase in wind speed becomes proportionately less effective in increasing the rate of CO_2 uptake as wind speed rises, and that above 100 m./min. (167 cm./sec.) it has no further effect.

A simpler graph is obtained by plotting, instead of time, the reciprocal of time; this is directly proportional to assimilation rate. The resulting plot for the same data (Fig. 1(*b*)) shows the points lying very near to a straight line. This line, if extrapolated back, cuts the vertical (assimilation) axis above zero, suggesting that a small amount of assimilation occurs when there is no wind; presumably diffusion and convection bring a limited supply of CO_2 to the leaves. Above this level, assimilation rate rises in direct proportion to wind speed up to the highest speeds investigated.

Fig. 1(*b*) also shows Deneke's results for *Thuja gigantea* and *Polytrichum*. The other species investigated (*Tradescantia pendula*, *Cereus tetragonus*, and *Avena sativa*) give graphs which lie between those of *Nerium* and *Polytrichum* but which have, for the sake of clarity, not been plotted. All are linear, showing a steady increase in assimilation rate as wind speed rises. The consistently good fit of the data is a tribute to Deneke's technique.

Though Deneke's investigation seems to be the only study of the effect of wind on CO_2 uptake, Heinicke and Hoffman (1933) have observed the effect of rate of air flow through small assimilation chambers on the rate of CO_2 uptake by apple leaves under greenhouse conditions. For high rates of air flow (2·5 to 7·5 l./sq. cm./hr.) they attached a small cup chamber of about 50 millilitres capacity to the underside of a leaf; for lower rates of flow they placed one or more leaves in a relatively close-fitting cellophane envelope.

Rates of air flow of 0·006 to 7·5 l./sq. cm./hr. were used and some idea of the wind speeds to which these may be expected to correspond can be obtained from their statement that a rate of flow of 2 to 2·5 l./sq. cm./hr. gave a mean air speed of about 7 m./min. (12 cm./sec.). Heinicke and Hoffman tabulate their results and conclude that '. . . it is clearly indicated that the assimilation rate falls off rapidly if the air supply is less than two liters per hour per square centimeter'. They make it clear that their results were obtained on different days and are therefore not strictly comparable, but even so their data, when plotted graphically, fall near to a smooth curve (Fig. 2(*a*)) which passes through the origin.

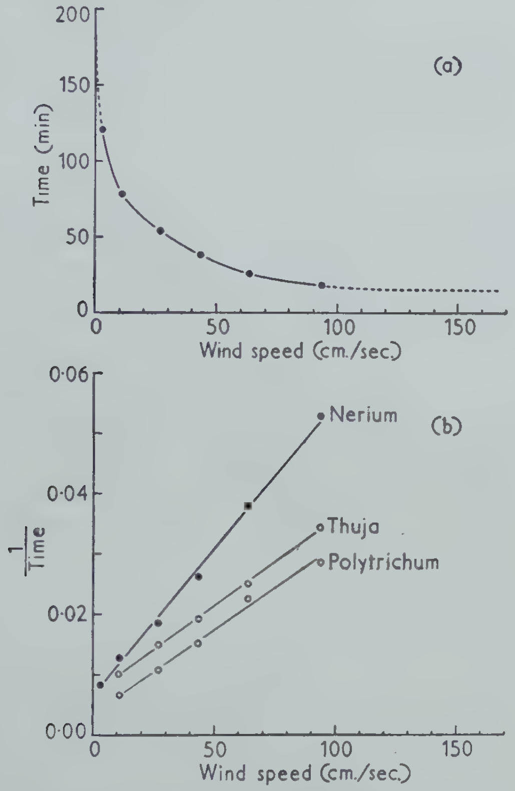


FIG. 1. (*a*) Deneke's graph (redrawn) for *Nerium*. The vertical axis measures the time taken for a standard amount carbon dioxide (CO_2) uptake. (*b*) The same data as in (*a*), but with the vertical axis measuring the reciprocal of time, i.e. assimilation rate. Deneke's results for *Thuja* and *Polytrichum* are also plotted.

On examining the results more closely we note that for each twofold increase in wind speed the uptake of CO_2 is nearly doubled. This observation is confirmed by the graph on double logarithmic scales (Fig. 2(*b*)) in which it is shown that the points lie near to a straight line and that a doubling of wind speed caused a 1·7 times increase in CO_2 uptake. Deneke's results (when treated so that the line passes through the origin) also approximate to a straight line on double logarithmic scales but because a doubling of wind speed doubles the CO_2 uptake a straight line is also obtained on linear scales. That Heinicke and Hoffman find a different proportionality constant from Deneke probably reflects the different experimental conditions.

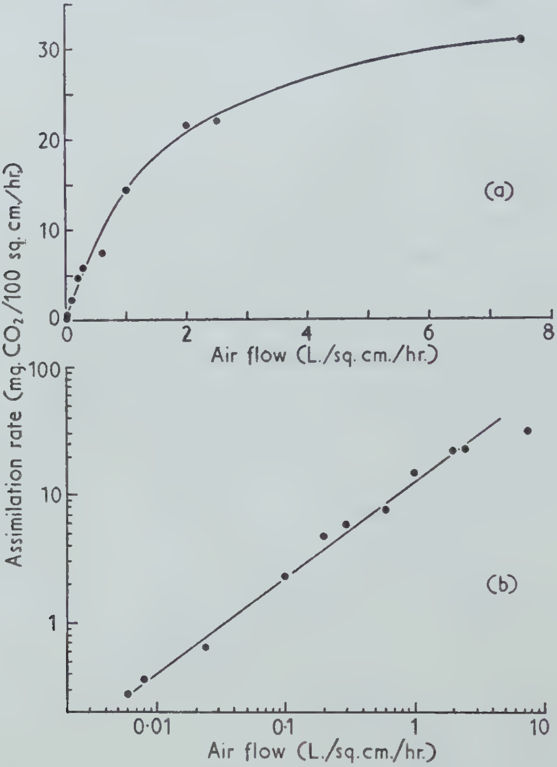


FIG. 2. Data of Heinicke and Hoffman for apple leaves, plotted on (*a*) linear scales and (*b*) logarithmic scales.

Thus under the experimental conditions used by Deneke and by Heinicke and Hoffman it can be shown that increase in air movement consistently accelerated CO_2 uptake over the entire range investigated.

Deneke's results show a roughly fivefold rise in assimilation rate resulting from increase in wind speed from 2 m./min. (3·3 cm./sec.) to 56 m./min. (93 cm./sec.). Much smaller effects than this would be significant for growth in the field, and it is worth inquiring whether wind speeds within vegetation normally lie in the range examined by Deneke. Only scattered information is available; the ranges of wind speed given below are mostly based on data collected on a few occasions and at various heights within each type of vegetation.

Wind speeds (in cm. per sec.) within Vegetation

Calluna heath	0·8 to 100 (Stocker, 1923)
Grassland	$<$ 5 to 200 (Stocker, 1933) 'practically zero' to 150 (Waterhouse, 1955)
Mountain summit vegetation	0·5 to 4 (Stocker, 1923) 1 to 40 (Warren Wilson, 1958)
Various crops	*c.* 10 to 100 (Kreutz, 1938; Ramdas, 1946) 'rarely exceeding' 130 (Broadbent, 1950)

No doubt stronger winds will affect shoots that are especially exposed (Stocker's value of 200 cm./sec. is for an open stand); but in general it seems that wind speeds in vegetation are of the same order as those used in Deneke's experiments.

On this basis it might be expected that the growth of plants in the field would normally be favoured by increase in wind speed. However, in those places where wind has any apparent effect on the growth of vegetation, it is in fact an adverse effect; moreover, all published experimental investigations of the effect of wind on plant growth (as measured by dry-weight increase) show decreased growth with increasing wind speed. This apparent conflict with Deneke's results is probably to be explained as follows.

The artificial winds used in growth experiments have been exceedingly strong. In the six published investigations the wind velocities used (for comparison with 'still' air) have ranged between 223 and 1,100 cm./sec.; and to the lowest speed must be added the speed of the natural wind, for the experiment took place out of doors. Assuming that the wind velocity within vegetation is around one-fifth of that in the open (Kreutz, 1938; Ramdas, 1946; Broadbent, 1950; Waterhouse, 1955), the plants in these experiments were subjected to wind speeds corresponding to wind speeds in the open of some 28 to 123 m.p.h., i.e. 'moderate gale' to 'hurricane'. Similarly, the effects of wind on plant growth in the field appear on cliffs and mountains, where winds are exceptionally strong.

At these high speeds harmful effects of wind which are not significant at the speeds and under the environmental conditions of Deneke's experiments may become important and mask the beneficial effect that he demonstrated. For example, wind adversely affects growth by tending to reduce the water status of plants (e.g. Martin and Clements, 1935). This effect will become increasingly potent as wind speed rises. In Deneke's experiments the drying action of the wind was minimized by deliberately keeping the air moist.

An experimental study of the effect of normal wind speeds on plant growth might therefore be expected to show that as wind speeds rise from zero to, say, 200 cm./sec., the growth-rate would at first increase (as a result of improved CO_2 supply) up to an optimum, and finally decrease (as drying and other harmful effects of higher wind speeds became increasingly important). Such a relationship has, in fact, been revealed in wind-tunnel experiments in this laboratory. The growth-rates of small stands of young *Brassica napus* plants exposed to continuous wind increased with rising wind speed up to an optimum at about 30 cm./sec., and decreased at higher speeds.

The optimal wind speed for growth will vary with species and environment. For this and other reasons the results of these wind-tunnel experiments are not directly applicable to vegetation in the field. Moreover, it may be difficult to measure the effect of wind on the assimilation rate of plants in their natural environment; but we consider it likely that within most vegetation, where wind speeds are low and moisture relations are adequate, acceleration of assimilation by wind occurs as it did in the controlled environment of Deneke's experiments.

ACKNOWLEDGEMENT

We are grateful to Professor T. M. Harris, F.R.S. for helpful criticism during the preparation of this paper.

LITERATURE CITED

BROADBENT, L., 1950: The Microclimate of the Potato Crop. Quart. J. R. met. Soc., **76,** 439–54.

DENEKE, H., 1931: Über den Einfluß bewegter Luft auf die Kohlensäureassimilation. Jb. wiss. Bot., **74,** 1–32.

HEINICKE, A. J., and HOFFMAN, M. B., 1933: The rate of Photosynthesis of Apple Leaves under Natural Conditions. Bull. Cornell agric. Exp. Sta., No. 577, pp. 32 ff.

KOSTYTSCHEW, S., BAZYRINA, K., and WASSILIEFF, G., 1927: Über die Kohlenstoffausbeute bei der Photosynthese unter natürlichen Verhältnissen. Biochem. Z., **182,** 79–87.

KREUTZ, W., 1938: Das Windschutzproblem. Bioklim. Beibl., **5,** 10–16.

MARTIN, E. V., and CLEMENTS, F. E., 1935: Studies on the Effect of Artificial Wind on Growth and Transpiration in *Helianthus annuus.* Plant Physiol., **10,** 613–36.

RAMDAS, L. A., 1946: The Micro-climates of Plant Communities. Indian Ecol., **1,** 1–20.

STOCKER, O., 1923: Klimamessungen auf kleinstem Raum an Wiesen-, Wald- und Heidepflanzen. Ber. dtsch. Bot. Ges., **41,** 145–50.

—— 1933: Transpiration und Wasserhaushalt in verschiedenen Klimazonen. II. Untersuchungen in der ungarischen Alkalisteppe. Jb. wiss. Bot., **78,** 751–856.

WARREN WILSON, J., 1958: Notes on Wind and its Effects in Arctic-alpine Vegetation. J. Ecol., **46,** (in press).

WATERHOUSE, F. L., 1955: Microclimatological Profiles in Grass Cover in Relation to Biological Problems. Quart. J. R. met. Soc., **81,** 63–71.

A Machine for Disintegrating Living Woody Shoots

BY

W. A. ROACH

(*East Malling Research Station, Nr. Maidstone, Kent*)

With Plate

Abstract

The disintegrator was constructed from a standard circular, tempered-steel wire brush. This was ground accurately to size and shielded for safety and to reduce draught. Each twig on being held against it was reduced to a fine powder that could be collected in a solvent. In this way chemical change was arrested.

THE disintegrator was designed and constructed in 1930 in an attempt to extract the organic chemical constituents of apple shoots as nearly as possible in the condition in which they exist in the living tissues. It had been found that one compound seen in microscope sections in the sub-epidermal cells as a bright red solution, was changed during the more usual methods of extraction into a brown insoluble substance (Roach, 1932). The machine consists of a shielded circular, tempered-steel wire brush rotating at 2,800 r.p.m. When shoots were held against this, they were reduced to a fine powder, which, when collected in a solvent, such as methyl alcohol, yielded a solution of the red compound in, as far as could be judged, an unaltered state. The machine has been in use ever since and a short description is put on record here in the hope that it may be of use to others.

The machine is illustrated in the Plate, in use in Fig. 2 at the bottom and with the shield at the top removed in Fig. 1 to show details of its construction. In the bottom right-hand corner of the Plate, Fig. 1, may be seen a circular wire-brush unit. This was a standard engineering unit consisting of crimped tempered steel wire of 33 S.W.G. threaded through holes around the circumference of a circular steel plate with a central hole of $\frac{1}{2}$-inch diameter. In the machine as illustrated, five of these were held between circular, light alloy plates of $5\frac{1}{2}$ inches external diameter on the spindle of a $\frac{1}{4}$ H.P. constant speed (2,800 r.p.m.) electric motor and compressed between the castle nut, seen in the illustration, and a shoulder on the motor spindle. When the nut was tightened as much as possible, it was secured with a split pin in the usual manner. The bristles of the brush protruded beyond the circumference of the end plates. The brush was rotated in an anti-clockwise direction and a piece of carborundum was held against the brush until the brush was of exactly the same diameter as the end plates. This was a tedious operation occupying some hours. Care had to be taken not to inhale the resulting carborundum dust nor to allow it to reach the moving parts of machinery, especially the bearings. The shield, seen in position in the lower part of the figure, was

[**Annals of Botany, N.S. Vol. 22, No. 86, 1958.**]

constructed from a cake-tin by cutting out the 'window' which occupied a quarter of the circumference. It was held in position by an iron bar bolted at each end to the board, as seen in the figure from which the top bolt has just been excluded. There was so little clearance between the tips of the bristles and the shield that time and care were necessary in the adjustment to avoid scratching of the shield by the bristles. As a result of this small clearance between the bristles and the shield, there was very little draught and consequently negligible loss of finely divided plant material.

The motor was provided with a reversing switch. For use the wheel was rotated in a clockwise direction.

The top of the vessel containing the solvent may be seen in both figures. The surface of the liquid solvent was about 1½ inches below the bottom of the shield. It was found that when the vessel was placed so that the torn off powder just fell inside the collecting vessel to the right, then little or no fine powder was carried by the draught beyond the left-hand margin of the liquid; but practically all fell into the liquid.

The end of each twig was held against the brush, as shown in Fig. 2 and fed forward with slow rotation to prevent the formation of 'streamers' of bark.

By removing the wheel from the spindle and mounting it the reverse way, the comparatively blunt side of each bristle comes in contact with the twig. When twigs were held gently tangentially, instead of radially, to the wheel, it was found possible to tear the bark off, separating it cleanly from the wood. The bark, however, was shredded rather than powdered and the decreased fineness of division resulted in a somewhat slower rate of extraction.

ACKNOWLEDGEMENTS

The machine was constructed by Mr. O. Usmar who then worked in the writer's laboratory. Mr. E. Yoxall Jones took the photographs.

LITERATURE CITEDS

ROACH, W. A., 1932: Chemical Examination of Apple Bark. Rep. E. Malling Res. Sta. for 1931, p. 35.

EXPLANATION OF PLATE

For description see text.

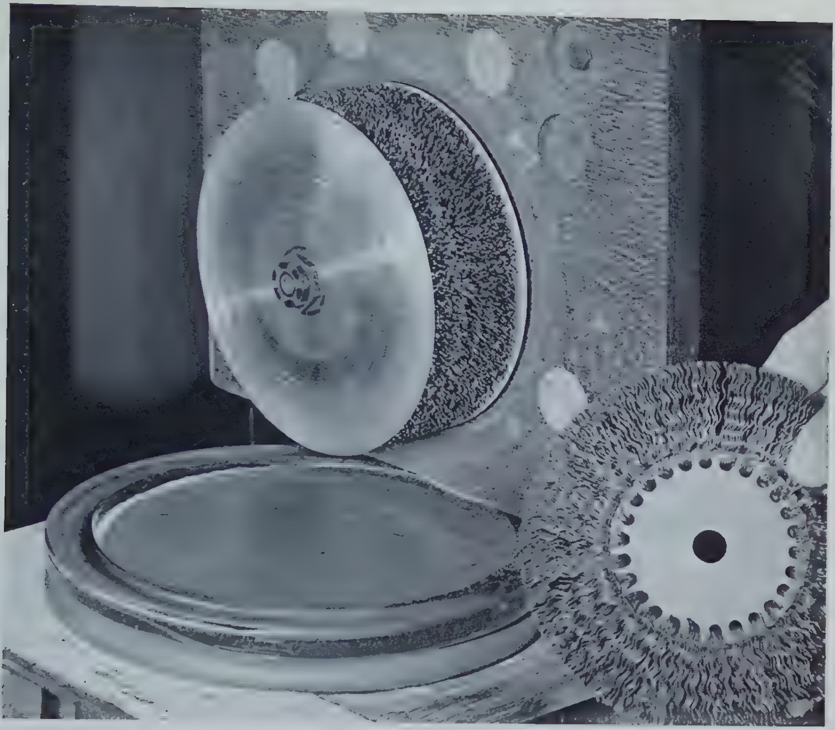


FIG. 1

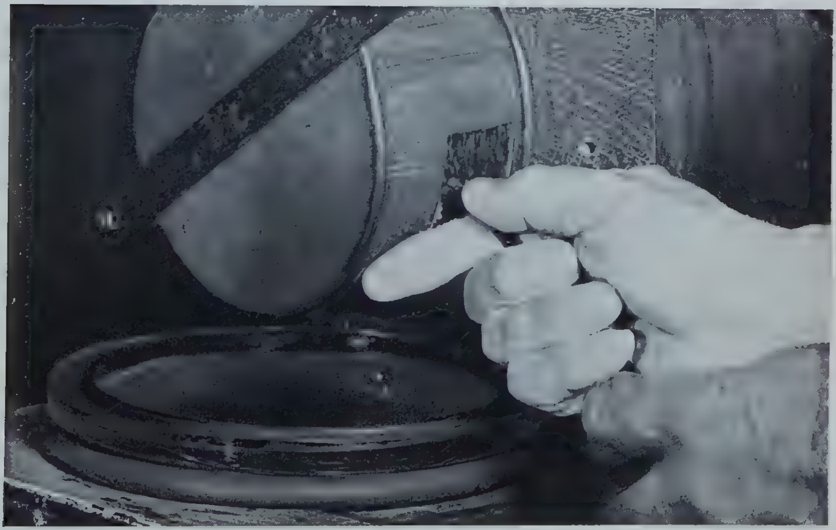


FIG. 2

W. A. ROACH

A Continuous Low-temperature Vacuum Extractor

BY

W. A. ROACH

(*East Malling Research Station, Nr. Maidstone, Kent*)

With Plate and one figure in the text

ABSTRACT

The extractor which was assembled mainly from standard laboratory apparatus, is suitable for kilogram quantities of plant materials.

THE extractor to be described is an improvement on one already described (Tattersfield and Roach, 1923). The Plate is from a photograph of it in its present form. The extraction takes place in the inverted bell jar 1. The solvent percolates down through the whole depth of plant material, flows along glass tube 3 and overflows through tubulure 7 into flask 8. Here it evaporates and the vapour rises through the insulated wide-bore tube 10 and is condensed in the condenser 12 and the condensed liquid falls, through the domed ground-on lid 15 of the bell jar, on the top of the material to be extracted. Necessary elasticity in this closed glass circuit is provided by standard spherical ground joints 2, 6, 11, and 14. A plug of cotton wool in the neck of the bell jar proves an efficient filter. This can be protected by placing on it a perforated porcelain disc and on this a circle of filter paper. In the illustration the plant material (leaves) is contained in a filter bag. Alternatively leaves may be dropped into the solvent in the bell jar and pressed down, or powder from the disintegrator, described in the previous article (Roach, 1958), may be collected directly in the bell jar. For convenience in photography two sides of the box surrounding the bell jar have been removed. When in use, of course, these are in position and the space between the bell jar and the sides of the box is packed with insulating material. Tap 4 is for drawing off the solvent at the end of an experiment. When tap 5 is closed, the extract in flask 8 may be concentrated and the solution may be drawn off through the tubulure provided on the opposite side of the flask to 9.

The inlet and outlet tubes of the condenser 12 are connected to the bottom and top respectively of an annular space surrounding the expansion chamber of an Electrolux electric refrigerator unit, and the whole filled with alcohol through the funnel. The whole of the cooling part is insulated with sponge-rubber sheet. The alcohol circulates naturally as a result of being warmed in the condenser, causing it to rise as a result of its expansion and consequent lowered density, and cooled in the annular space round the expansion chamber, causing it to fall as a result of contraction and consequent increased density. The cooling water from the electric refrigerator unit is passed through the water bath, in which flask 8 is partly immersed, and so acts as

[Annals of Botany, N.S. Vol. 22, No. 86, 1958.]

a source of heat for the evaporation of the solvent. The temperature of the water in the bath is usually about 30° C. and that of the solvent in flask 8 about 20° C.

The system is evacuated through tap 13. If evacuated with the water pump over-night, the solvent is usually still circulating in the morning but slowly. It is found advantageous, therefore, to maintain the vacuum constant by means of a small auxiliary pump. A mercury drop pump 16 serves this purpose well. The most convenient way of raising the mercury to the top of the drop pump was found to be by means of a small rotary gear pump such as is fitted to motor bicycles for circulating oil if one can be procured or made entirely of steel; but when difficulty was experienced in replacing such a pump, the one partly seen in the Plate and shown in section in Fig. 1 was constructed of glass hypodermic syringes, perspex, and polythene. A small electric motor operates the linked pistons of two all-glass hypodermic syringes. The one on the right is sucking mercury down from the reservoir past the polythene sheet valve while mercury is prevented from rising in the bottom chamber 3 by another polythene sheet valve. At the same time the piston on the left is forcing mercury past the polythene sheet valve in the bottom chamber 4, up the central channel 6, and thence up the one arm of the drop pump (see Plate), at the top of which it falls in droplets, each of which carries gas and vapour in front of it down the other tube, the bottom of which, 7, is seen in Fig. 1, and the gas and vapour bubble up through the mercury in the reservoir. The central part of the pump was assembled from plates of perspex, some of which were stuck together after moistening with chloroform, while others were bolted together, 0·005 inch polythene sheet being used as gasket material.

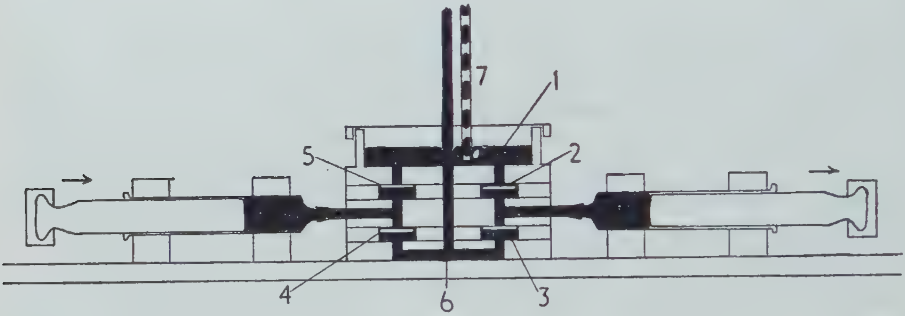


FIG. 1.

Some, as yet unidentified, vapour from the plant material appeared to contaminate the mercury to such an extent as to interfere with the working of the pump when rigid perspex valves were used instead of the softer polythene ones. A glass tube cooled in solid carbon dioxide was, therefore, introduced between tap 13 and the mercury drop pump in an attempt to trap this substance.

To prevent 'bumping' in flask 8, nitrogen was allowed to leak through a capillary tube dipping under the surface of the liquid. The wide part of this tube passed through tubulure 9.

ACKNOWLEDGEMENTS

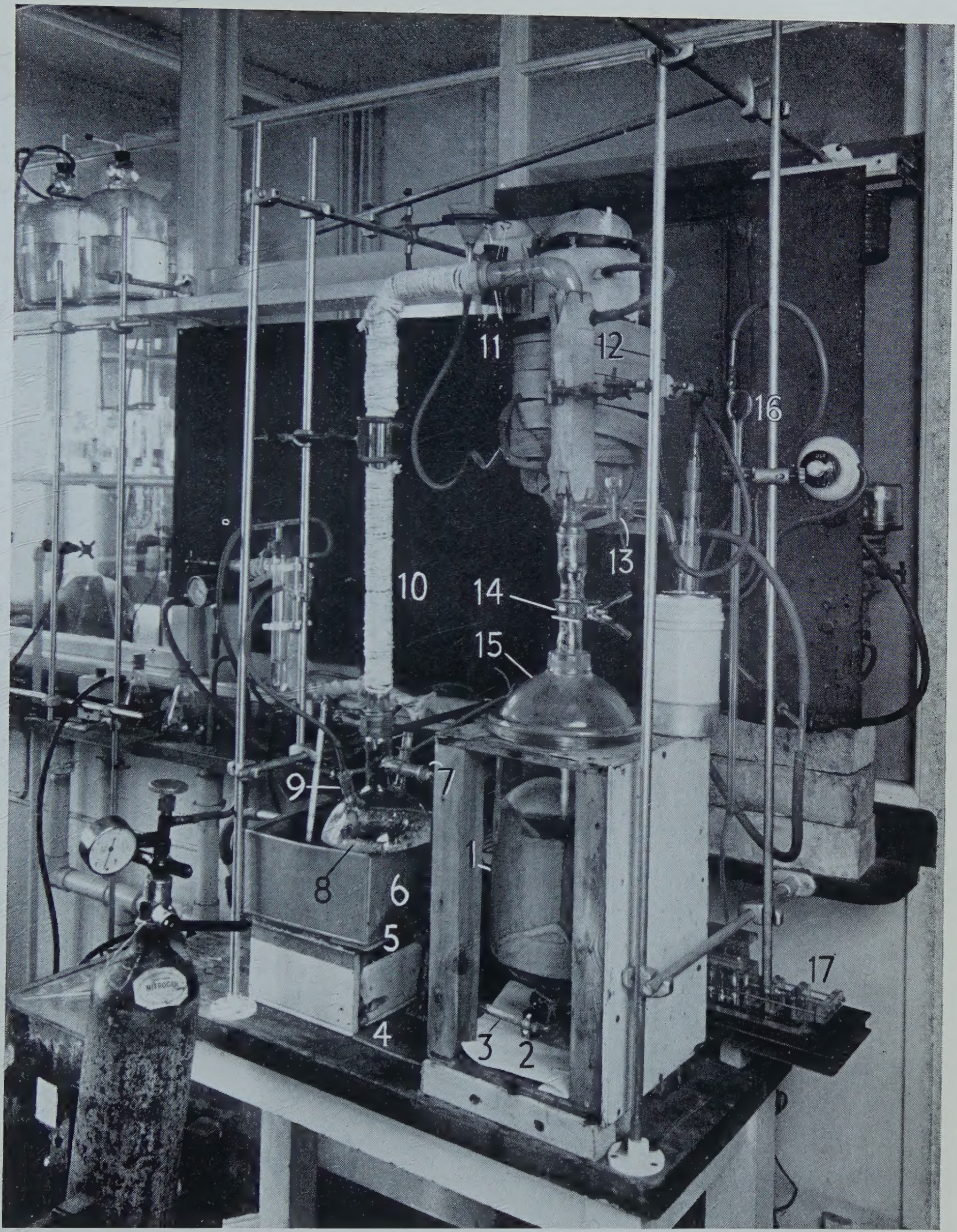
The mercury pump was constructed to the writer's design by Mr. R. Neve. The writer also acknowledges gratefully suggestions made by Mr. H. Hutchins as a result of his experience gathered during use of the apparatus. Mr. E. Yoxall Jones took the photograph.

LITERATURE CITED

TATTERSFIELD, F., and ROACH, W. A., 1923: The Chemical Properties of *Derris elliptica* (Tuba Root). Ann. appl. Biol., **10,** 1–17.

EXPLANATION OF PLATE

For description see text.



W. A. ROACH

THE JOURNAL OF ECOLOGY

EDITORS: D. E. COOMBE AND P. W. RICHARDS

VOL. 46, No. 1 FEBRUARY 1958

Sir Arthur Tansley, F.R.S.—Obituary.

Ecological studies on growth-form in bryophytes. III. The relationship between the growth-form of mosses and ground-water supply. By EVELYN M. BIRSE.

Ecological studies on growth-form in bryophytes. IV. Growth-form distribution in a deciduous wood. By EVELYN M. BIRSE.

A mixed teak forest of Central India. By K. K. BHATIA.

Light intensity measurements in rain forest near Santarem, Brazil. By P. S. ASHTON.

The hardness of the soil of the shore at Whitstable, Kent. By E. J. PERKINS.

Movement of vegetated sand dunes at Newborough Warren, Anglesey. By DEREK RANWELL.

Changes in the weed flora on Broadbalk permanent wheat field during the period 1930–1955. By KATHERINE WARRINGTON.

Some edaphic vegetational types at Kiboko, Kenya. By A. V. BOGDAN.

Studies of the development of woodland conditions under different trees. VI. Soil sodium, potassium and phosphorus. By J. D. OVINGTON.

The radiation error of air temperature measurements by Stantel Type F thermistors and simple methods to reduce it. By B. N. BOWDEN.

The survival of fresh water algae during dry periods. I. An investigation of the algae of five small ponds. By JOHN H. EVANS.

Sheep population and environment in Snowdonia (North Wales). By R. ELFYN HUGHES.

Dirt on snow patches. By J. WARREN WILSON.

Reviews: Biological Flora of the British Isles.

Articles are illustrated with plates and text-figures

The subscription price per volume of 3 parts is 84s. net, single parts 35s. net

BLACKWELL SCIENTIFIC PUBLICATIONS LTD.

24–25 BROAD STREET · OXFORD

Printed in Great Britain at the University Press, Oxford, by Charles Batey, Printer to the University